PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Commissioner
US Department of Commerce
United States Patent and Trademark
Office, PCT
2011 South Clark Place Room
CP2/5C24
Arlington, VA 22202
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing: 01 March 2001 (01.03.01)	
International application No.: PCT/AU00/00982	Applicant's or agent's file reference: 101540
International filing date: 17 August 2000 (17.08.00)	Priority date: 19 August 1999 (19.08.99)
Applicant: LITTLE, David, G.	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International preliminary Examining Authority on:

23 November 2000 (23.11.00)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No.: (41-22) 740.14.35

Authorized officer:

J. Zahra

Telephone No.: (41-22) 338.83.38

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
1 March 2001 (01.03.2001)

PCT

(10) International Publication Number
WO 01/13922 A1

(51) International Patent Classification[7]: A61K 31/663, A61P 19/00

(21) International Application Number: PCT/AU00/00982

(22) International Filing Date: 17 August 2000 (17.08.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
PQ 2325 19 August 1999 (19.08.1999) AU

(71) Applicant *(for all designated States except US)*: **THE ROYAL ALEXANDRA HOSPITAL FOR CHILDREN** [AU/AU]; Cnr Hawkesbury Road & Hainsworth Street, Westmead, NSW 2145 (AU).

(72) Inventor; and
(75) Inventor/Applicant *(for US only)*: **LITTLE, David, G.** [AU/AU]; Suite 3, Children's Hospital Medical Centre, Hainsworth Street, Westmead, NSW 2145 (AU).

(74) Agent: F B RICE & CO; 605 Darling Street, Balmain, NSW 2041 (AU).

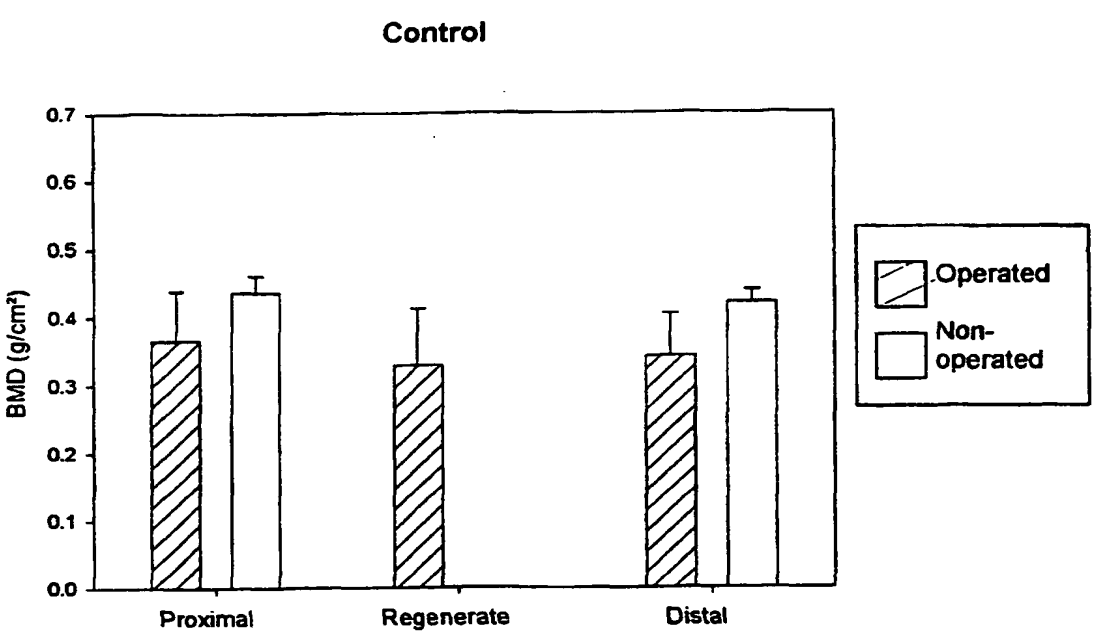
(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:
— *With international search report.*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: DRUG FOR TREATING FRACTURES



(57) Abstract: A drug selected from a group consisting of bisphosphonates for promoting bone growth and for the treatment of a fracture.

WO 01/13922 A1

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU00/00982

A. CLASSIFICATION OF SUBJECT MATTER

Int. Cl. [7]: A61K 031/663, A61P 19/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K 031/66

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
AU:IPC AS ABOVE

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
WPAT, MEDLINE; keywords: bisphosphonate, Zoledronate, Pamidronate, bone, fracture, skelet:.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 98/00438A (THE UNIVERSITY OF LIVERPOOL) 8 January 1998. See whole document.	1-20
X	WO 96/39150A (MERCK & CO., INC) 12 December 1996. See whole document.	1-20
X	WO 96/39151A (MERCK & CO., INC) 12 December 1996. See whole document.	1-20
X	WO 95/28936A (MERCK & CO., INC) 2 November 1995 . See whole document.	1-20

[X] Further documents are listed in the continuation of Box C [X] See patent family annex

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search
21 September 2000

Date of mailing of the international search report
26 SEP 2000

Name and mailing address of the ISA/AU

AUSTRALIAN PATENT OFFICE
PO BOX 200, WODEN ACT 2606, AUSTRALIA
E-mail address: pct@ipaustralia.gov.au
Facsimile No. (02) 6285 3929

Authorized officer

G.R.PETERS
Telephone No : (02) 6283 2184

Form PCT/ISA/210 (second sheet) (July 1998)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU00/00982

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 94/21266A (LEIRAS OY) 29 September 1994. See whole document.	1-20
X	WO 93/11786A (PROCTER & GAMBLE PHARACEUTICALS, INC.) 24 June 1993. See whole document.	1-20
X	Taxel P, "Osteoporosis: Detection, prevention and treatment in primary care." Geriatrics Vol 53 no 8, Aug 1998 pages 22-40	1-20
X	Eck, J et al, " Diagnosis and Treatment of Common Metabolic Spinal Disorders in the Geriatric Population." Southern Medical Journal Vol 91 no 12, Dec 1998 pages 1089-1097.	1-20
X	Sabokar, A et al, "Bisphosphonates in bone cement inhibit PMMA particle induced bone resorption." Annals of Rheumatic Diseases Vol 57 no 10, Oct 1998 pages 614-618.	1-20

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/AU00/00982

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report	Patent Family Member				
WO 98/00438	AU 33506/97	BG 103123	BR 9710074	CN 122625	CZ 9804323
	EP 912598	GB 9613722	HU 9902880	ZA 9705744	
WO 96/391450	AU 59679/96	CA 2221416	EP 831843		
WO 96/39151	AU 60309/96	CA 2223400	EP 831844	IL 122149	US 5616571
WO 95/28936	AU 234748/95	BG 100910	CA 2188030	CN 1146152	EP 756483
	HU 9602888	NO 964441	US 5646134		
WO 94/21266	AU 62093/94	EP 689443	US 5403829		
WO 93/11786	AU 30226/92	BR 9206941	EP 618805	RU 2104699	US 5616560
	ZA 9209758				

END OF ANNEX

10/049556
PCT/AU00/00982
IC13 Rec'd PCT/PTO 12 FEB 2002

30/prts

Drug for Treating Fractures

Field of the Invention

The present invention relates to new indications for a certain class of drugs. More specifically, the present invention relates to the use of bisphosphonates for the promotion of bone growth and in the treatment of bone fractures. Of the bisphosphonates, Zoledronate and Pamidronate have been found to be particularly effective when used for such a purpose.

Background Art

Bisphosphonates are characterised by a P-C-P bond, which has a strong affinity for bone mineral. They are analogues of pyrophosphate, containing a carbon instead of an oxygen atom. This makes them totally resistant to enzymatic breakdown in vivo.

Bisphosphonates differ in their actions and potency depending on the configuration of a side chain. Bisphosphonates inhibit bone resorption through a direct effect on osteoclast function, and also inhibit osteoblastic recruitment of osteoclasts. Due to these factors, calcium is retained in the skeleton and there is a subsequent increase in parathyroid hormone (PTH) and 1,25-$(OH)_2$ vitamin D, leading to increased intestinal calcium absorption. In growing rats this has resulted in an increase in bone mass (Licata, "Bisphosphonate Therapy" *Am J Med Sci* 1997 Jan; 313(1):17-22). In very high doses, bisphosphonates may actually inhibit bone formation and osteoblast function. In fact one of the previously well-documented indications for bisphosphonates is in the prevention of heterotopic ossification after spinal cord injury or hip arthroplasty. (Stover et al "Disodium etidronate in the prevention of postoperative recurrence of heterotopic ossification in spinal-cord injury patients" *J Bone Joint Surg Am* 1976;58(5):683-8 and Finerman and Stover "Heterotopic ossification following hip replacement or spinal cord injury. Two clinical studies with EHDP." *Metab Bone Dis Relat Res* 1981;3:337-42 and Banovac and Gonzalez "Evaluation and management of heterotopic ossification in patients with spinal cord injury". *Spinal Cord* 1997;35:158-62.)

The bisphosphonate etidronate, was first trialed for the treatment of primary osteoporosis many years ago. Results showed some success in increasing bone density and possibly controlling fracture rates (Licata, *supra*).

Since then, the use of bisphosphonates in the treatment and

prevention of osteoporosis has become well known. They are particularly valuable in the management of postmenopausal osteoporosis but indications for the use of these drugs in the treatment of other disorders affecting bones has also become well known.

Because bisphosphonates are capable of inhibiting bone resorption they are used as effective therapeutic agents in several conditions characterised by increased bone turnover, including Paget's disease, hypercalcaemia of malignancy and metastatic bone disease (Lombardi, "Clinical trials with bisphosphonates" *Ann Ital Med Int* 1992 Jul-Sep; 7(3 Suppl):158S-165S). They have also been indicated in the treatment of multiple myeloma, breast cancer metastases and osteogenesis imperfecta.

Bone fractures are often par for the course in many of these disorders. Even where fractures are avoided, however, their risk of occurrence is dramatically increased by the presence of such disorders. Consequently, much of the ongoing research into the use of bisphosphonates in treating these disorders has centred around the safety of continuing such treatment following a revealed fracture. Many of these studies have found that bisphosphonates have no adverse effects on the restoration of the mechanical integrity of a long bone after fracture or on fracture healing (see, for example, Goodship et al "Use of a bisphosphonate (pamidronate) to modulate fracture repair in ovine bone" *Ann Oncol* 1994; 5 Suppl 7:S53-5; see also Li et al "Effect of Bisphosphonate (Incadronate) on Fracture Healing of Long Bones in Rats" *J Bone Miner Res* 1999 June; 14(6):969-79).

Although the use of bisphosphonates in the above mentioned and other disorders is well documented, "their mode of action is still being unravelled. As a result, their full therapeutic potential is gradually being realised" (see abstract, Russell et al, "Bisphosphonates: from the laboratory to the clinic and back again" *Bone* 1999 Jul; 25(1):97-106).

The present invention provides a considerable number of novel and important indications for the administration of bisphosphonates.

Description of the Invention

In a first aspect, the present invention consists in a drug selected from a group consisting of at least one bisphosphonate when used for promoting bone growth.

Preferably, bone growth is promoted at a fracture site. Furthermore, it is envisaged that bone growth is promoted between a bone and a prosthesis.

bone fixation device or any other bone or dental implant.

In a second aspect, the present invention consists in a drug selected from a group consisting of at least one bisphosphonate when used for treating a fracture.

In a preferred embodiment, the drug of either the first or the second aspect is the bisphosphonate Zoledronate.

In a further preferred embodiment, the drug of either the first or the second aspect is the bisphosphonate Pamidronate.

In another embodiment, the drug may be another drug from the group consisting of bisphosphonates or a combination of two or more bisphosphonates.

In a third aspect, the present invention consists in the use of a drug selected from a group consisting of at least one bisphosphonate for the manufacture of a medicament for promoting bone growth.

In one embodiment of the third aspect, the drug promotes bone growth at a fracture site.

In a further embodiment, the drug promotes bone growth between a bone and a prosthesis.

In a fourth aspect, the present invention consists in the use of a drug selected from a group consisting of at least one bisphosphonate for the manufacture of a medicament for treating a fractured bone.

In a fifth aspect, the present invention consists in a method for treating a fractured bone, the method including administering to a subject with a fractured bone a therapeutically effective amount of a drug selected from the group consisting of at least one bisphosphonate.

Preferably, the drug is administered to the subject as a single dose. It is further preferred that the single dose of drug is administered at an early stage of treatment of the fractured bone.

In a sixth aspect, the present invention consists in a method for treating a fractured bone, the method including the steps of:

(a) administering to a subject with a fractured bone a therapeutically effective amount of a drug selected from a group consisting of at least one bisphosphonate; and

(b) providing a vibratory stimulus to the fractured bone.

Preferred embodiments disclose that the use of a drug from the class of bisphosphonates in promoting bone growth or treating a fracture may be

applicable to all clinical alternatives for managing a fractured bone: many fractures are most appropriately managed by simply applying a plaster cast to the site at which the fracture has occurred and allowing the bone to heal whilst splinted in that way; some fractures simply require the patient to rest; some fractures may require the application of a range of surgical interventions; and other fractures are appropriately managed with a combination of the latter two alternatives. Furthermore, as an alternative or an addition, as disclosed above, the step of providing of a vibratory stimulus to the fractured bone may also be desirable in certain circumstances. Which ever of these, or other, clinical alternatives is/are chosen for managing a fractured bone, the present invention discloses the administration of at least one drug from the class of bisphosphonates to the patient.

There may be several advantages to using at least one drug from the class of bisphosphonates in the treatment of bone fractures. Many of these are disclosed in considerable detail below. Nevertheless, for reasons which will now become clear, it is worthwhile noting some of the more general advantages from the outset. They include, but are not limited to, the following: bisphosphonates can stimulate osteoblast proliferation and increase callus formation; they are also potent inhibitors of osteoclastic bone resorption; they aid in the prevention of osteoporosis, and therefore decrease disuse osteoporosis associated with the injury; and they may also significantly decrease the length of time which is taken for a fracture to heal.

According to this invention, the circumstances in which bisphosphonates are applicable to the management of fractures are far reaching. Indeed, preferred embodiments disclose that the situations in which bisphosphonates may be indicated in fracture care include at least:

(i) Increasing new bone formation in distraction osteogenesis:
  (a) Bone lengthening
  (b) Bone transport
(ii) Increasing new bone formation in fractures treated by open reduction
(iii) Increasing new bone formation in fractures treated by intramedullary fixation
(iv) Increasing new bone formation in fractures treated by external fixation
(v) Delayed union

(vi) Improving the osteogenic potential of bone graft – autologous graft, allograft or synthetic bone graft substitute

(vii) Improving the ability of bone to support internal fixation devices in osteoporotic individuals or in locally osteoporotic bone segments

(viii) Treating fractures in which there are potential impediments to uncomplicated healing, for example:

(a) Fractures in the elderly including: neck of femur; supracondylar femur; tibia; ankle; humerus; and the distal radius (note that this list merely provides examples, and the use of bisphosphonates according to this invention is not by any means limited to treating these fractures only, or any other fractures, for that matter, in people of all ages)

(b) Pubic rami fatigue fracture

(c) Pathological fracture

(d) Scaphoid fracture

(e) Open fracture

(f) Fracture with periosteal disruption

(ix) Treating fractures that require prolonged immobilisation when treated non-operatively, for example: femoral fractures, tibial fractures; and fractures of the foot and ankle.

(x) Treatment of patients with avascular necrosis to enhance new bone formation and prevent collapse

(xi) Treatment of congenital pseudarthrosis of the tibia and related conditions.

Further preferred embodiments also disclose a number of additional indications for using bisphosphonates in orthopaedic procedures. These include administering bisphosphonates to increase ingrowth of bone into joint replacement prostheses; and coating joint prosthesis with bisphosphonates to enhance the latter mentioned ingrowth at a more local level. Such therapy should also reduce the effects of periprosthetic stress shielding. Prosthetic implants may be so coated as an alternative, or in addition to coating with hydroxyapatite or some other osteoinductive coating.

Furthermore, bisphosphonates may be used in arthrodesis, that is,

fusing of a joint to increase the fusion rate.

One preferred embodiment discloses that the drug chosen from the class of bisphosphonates for carrying out this invention is Pamidronate. Another preferred embodiment discloses that the drug chosen from the group is Zoledronate. However, in further preferred embodiments, other bisphosphonates may be used in addition (where no adverse interaction results), or as an alternative, to Pamidronate or Zoledronate. Examples of further bisphosphonates include, but are not limited to, Alendronate, Tiludronate, Risedronate, Ibandronate and Incadronate.

In further preferred embodiments, the drug is administered to a patient as a single dose. In this embodiment, it is preferred that the administration of the drug occurs early during the course of treating the fractured bone as administration of a bisphosphonate at such an early stage has a positive effect on the stimulation and proliferation of osteoblasts.

In a further embodiment, subsequent additional doses may be administered to the patient. In this embodiment, it is envisaged that a response to the first dose would be assessed before administering additional doses.

In still further preferred embodiments, the mode of administration may be as a perioperative intravenous infusion, orally, transdermally or by some other route. Alternatively a course of an oral bisphosphonate may be prescribed. All preferred and alternative embodiments of the invention envisage current and future available modes of administration for the drug. Such modes of administration must, of course be plausible, convenient and provide the patient with a therapeutically effective dose for treating and/or promoting healing of the fractured bone.

The present invention also discloses that in some embodiments, it is preferable to additionally apply a vibratory stimulus to the fractured bone. The vibratory stimulus may be provided by ultrasound stimulation and vibration stimulation, or any other mechanism and/or device capable of providing vibratory stimulation. In some embodiments, the vibratory stimulus may be applied at any frequency which is considered to be effective in the treatment of a fractured bone. In preferred embodiments, however, the step of providing a vibratory stimulus includes periodically providing a vibratory stimulus at the resonant frequency of the bone, said resonant frequency being calculated as a function of the bone's vibratory response to

the vibratory stimulus. In order to have this achieved, this step may be broken down into the following components:

(a) providing a vibratory stimulus to the fractured bone:

(b) detecting the vibratory response of the bone to the vibratory stimulus:

(c) generating a signal representative of the vibratory response:

(d) processing the signal to identify at least one resonant frequency of the bone; and

(e) providing a signal to adjust the vibratory stimulus to the bone such that it is at, or approximate, the bone's at least one resonant frequency.

Whether or not the step of providing a vibratory stimulus to the fractured bone is utilised, preferred embodiments disclose that at least one bisphosphonate should be administered to the patient early in the course of treatment. In this regard, different bones and different types of fracture heal at varying rates. Accordingly, the early phase of the course depends upon such variables.

The administration of a bisphosphonate at such an early stage has a positive effect on the stimulation and proliferation of osteoblasts. In cases where the provision of a vibratory stimulus is utilised, it is preferable that such stimulus be provided later in the course of treatment, since mechanical stimulation will assist in the maturation of the healing fracture. In yet another preferred embodiment, however, both the administration of a bisphosphonate and the provision of a vibratory stimulus may occur early in the course of treatment. In alternative embodiments, they may be used at opposite times, they may be alternated, or they may both be delivered in the later stages of treatment as is considered to be most appropriate.

Brief Description of the Drawings

By way of example, preferred embodiments of the invention are described with reference to the accompanying drawings in which:

Fig. 1 shows the generic formula for bisphosphonates.

Fig. 2 is a graph from example 1 illustrating the differences in Bone Mineral Density (BMD) at the regenerate and at locations both proximal and distal the regenerate in the control group with lengthened legs and the control group with non-lengthened legs (there is, of course, no value given for the BMD at the regenerate for the group with non-lengthened legs):

Fig. 3 is a graph from example 1 illustrating the differences in BMD at

the regenerate and at locations both proximal and distal the regenerate in the pamidronate treated group with lengthened legs and the pamidronate treated group with non-lengthened legs (there is, of course, no value given for the BMD at the regenerate for the group with non-lengthened legs);

Fig. 4 is a graph from example 1 illustrating the differences in BMD at the regenerate and at locations both proximal and distal the regenerate in the control group with lengthened legs and the pamidronate treated group with lengthened legs;

Fig. 5 illustrates the histopathological differences between a specimen from the control group and one from the pamidronate treated group of example 1;

Fig. 6 is a graph from example 2 illustrating the differences in peak load for the non-operated and operated pamidronate treated group and for the non-operated and operated control group; and

Fig. 7 is a graph from example 2 illustrating the difference in findings with respect to Young's Modulus (1% strain) for the non-operated and operated pamidronate treated group and for the non-operated and operated control group.

Figures 8A, 8B and 8C are graphs from example 3 illustrating the bone mineral content in the proximal, regenerate and distal segments of an operated tibia at 2, 4 and 6 weeks post operation respectively.

Figure 9 is a graph from example 3 illustrating Bone Mineral Content (BMC) accrual in the regenerate.

Figure 10 is a graph from example 3 illustrating final BMC at six weeks as measured by QCT.

Figures 11A, 11B and 11C are graphs from example 3 illustrating BMD in the proximal, regenerate and distal segments of an operated tibia at 2, 4 and 6 weeks post operation respectively.

Figure 12 is a graph from example 3 illustrating final BMD at six weeks as measured by QCT.

Figure 13 is a graph from example 3 illustrating final cross-sectional area at six weeks as measured by QCT.

Figure 14 depicts QCT scans from example 3 of regenerate in rabbit operated tibiae that were the median for cross sectional area in each group.

Figure 15 is a graph from example 3 illustrating final moment of inertia at six weeks as measured by QCT.

Figure 16 is a simplified view of a device for the application of a vibratory stimulus to a fractured bone.

Preferred Mode of Carrying Out the Invention

In preferred embodiments, zoledronate or pamidronate and/or another drug from the class of bisphosphonates is used for the manufacture of a medicament for promoting bone growth or treating a fractured bone.

A therapeutically effective dose of the medicament, or zoledronate or pamidronate and/or another bisphosphonate alone, is then appropriately prepared and administered to a patient via an intravenous route.

Preferred embodiments further disclose that such administration of the drug is to occur early during the course of treating the fractured bone. The administration of a bisphosphonate at such an early stage has a positive effect on the stimulation and proliferation of osteoblasts. No further administration of the bisphosphonate may be required, but can be administered if desirable after gauging the response of the patient to the first dose.

Furthermore, a course of an oral bisphosphonate may be prescribed to a patient wherein the oral bisphosphonate is taken in the initial three months of fracture healing. In another embodiment, a course of oral bisphosphonates may be given later in the course of fracture healing to augment callus formation in a bone healing slowly.

The present invention also discloses that in some embodiments, it is preferable to additionally apply a vibratory stimulus to the fractured bone as set out in International Application No PCT/AU99/00974 and herein incorporated by reference. The vibratory stimulus may be provided by ultrasound stimulation and vibration stimulation, or any other mechanism and/or device capable of providing vibratory stimulation. In such preferred embodiments, the step of providing a vibratory stimulus includes periodically providing a vibratory stimulus at the resonant frequency of the bone, said resonant frequency being calculated as a function of the bone's vibratory response to the vibratory stimulus.

With reference to Figure 16, the vibratory stimulation device 10 is adapted to determine the specific resonant frequency of a bone 11, and to then subject the bone 11 to stimulation at the specific resonant frequency of the bone 11, and maintain stimulation at that frequency for a period of time. This ensures optimal stimulation and thus optimal promotion of bone mass.

whilst at the same time, avoids the risk of over-stimulation or overloading, and thus, fracture of the bone.

The device 10 includes a vibration stimulator 12 which, when activated, stimulates the bone 11 over a range of frequencies, causing vibration of the bone 11. The stimulator 12 is driven by a signal generator housed in the computer 14. The signal generator, when initially activated, can cause the stimulator 12 to vibrate over a range of frequencies. The signal generator can, for example, sweep through this range of frequencies.

Examples of suitable stimulators 12 include a rotating eccentric mass, an electromagnetic shaker, and a variable frequency pulsed ultrasonic transducer. The stimulator 12 can incorporate a stimulus sensor, such as a force transducer, to monitor the stimulus provided to the bone by the stimulator 12.

The vibrations are detected by a detector 13. The detector 13 can comprise an accelerometer or a plurality of accelerometers. The detector 13 transmits the signals to a computer 14 wherein the signals are converted from analogue to digital form and then processed to determine the frequency domain characteristics of the vibratory response. The computer 14 can incorporate an automatic analysing means that determines the peak acceleration/velocity/displacement of the bone and so determines the resonant frequency of the bone. In another embodiment, the computer can display either numerically and/or graphically the measured characteristics of the vibratory response to allow manual determination of the resonant frequency by a user of the device, or a treating physician. The device 10 can include a manual frequency control 15 for stimulation. Preferably, from the signals received, the computer 14 identifies one of the resonant frequencies of the bone 11 and transmits a signal to the stimulator 12 to stimulate the bone 11 at, or approximate, the one resonant frequency.

The device can incorporate a timer that allows the time of operation of the stimulator 12 to be pre-set prior to activation. Such a time might be set by making an appropriate entry into a software programme running on the computer 14. Preferably, adjustment to the amplitude of the vibratory stimulus can be made by suitable entries into the software running on the computer 14.

Whilst Figure 16 depicts a simple representation of the device whereby the vibration stimulator 12 and the detector 13 are applied to the bone via an

external frame 16, it is envisaged that they would be applied either to the skin of the affected limb, wherein the stimulation of the bone could be imparted transcutaneously, or via a frame, or other like device surrounding the limb, and in particular, a plaster cast.

It is further envisaged that this method of treatment be used in the treatment of several bone disorders to promote bone tissue growth and also to maintain bone mass. Examples include the healing of a fracture site wherein the vibration caused to the bone results in micro-movement, bending or torsion at the site of fracture which in turn leads to promotion of bone healing. The stimulation also causes micro-movement, bending or torsion of the intact portions of the bone, which in turn, leads to promotion of bone formation and prevention of osteoporosis in the intact bone. As the stimulation imparted by device 10 is regulated to be at the same, or approximately the same, frequency as the resonant frequency of the bone, the promotion of bone tissue growth is optimised and occurs at a faster rate than if the bone is simply stimulated at a frequency unrelated to the resonant frequency of the bone.

Similarly, it is readily envisaged that the device 10 could be applied transcutaneously to a bone with a fixation means such as an intramedullary nail holding the bone pieces together. In this manner, the formation of bone between the pieces of bone may be increased by the stimulation of the bone and the fixation means at, or approximate, the bone's resonant frequency.

It can also be envisaged that the device 10 can be transported readily and therefore used in a patient's home. In this way, the device 10 would be pre-programmed such that all the patient need do to use the device would be to attach the vibration stimulator 12 and the vibration detector 13 to the affected limb, or other body part, and activate the device 10.

In cases where the provision of a vibratory stimulus is utilised, it is preferable that such stimulus be provided later in the course of treatment, since mechanical stimulation assisting in the maturation of the healing fracture. In yet another preferred embodiment, however, both the administration of a bisphosphonate and the provision of a vibratory stimulus may occur early in the course of treatment. As disclosed above, however, these two modes of treatment may be used at opposite times, they may be alternated, or they may both be delivered in the later stages of treatment as is considered to be most appropriate.

The foregoing disclosure will now provide a more detailed analysis of the effects which the administration of a drug according to a preferred embodiment of the invention has on the healing process, and therefore, treatment of a fractured bone.

Each of the following examples relates to the administration of a bisphosphonate in distraction osteogenesis. One of the aims of distraction osteogenesis is to lengthen the limb upon which the procedure is performed. The performance of a distraction osteogenesis necessitates fracturing of the bone. Following placement of an external frame on the limb, the bone is cut. During healing, the new bone forms as the frame is slowly distracted.

It is because the bone is fractured during the procedure, that the results of a study involving distraction osteogenesis are appropriate for illustrating the effects which the administration of pamidronate has on bone.

Example 1: Pamidronate in Distraction Osteogenesis (dose 3mg/kg)

Methods:

Experimental design

Twenty eight-week-old male NZW rabbits underwent tibial lengthening. Similar rabbit models have been reported. After premedication with IM Ketamine 15 mg/kg and Xylazine 4mg/kg, anaesthesia was administered with Halothane 2%, and Oxygen 1 l/min. An open mid-tibial drill hole osteotomy was performed on each rabbit and an Orthofix M-100 fixator was applied using four Orthofix 3 mm half pins (Orthofix, Bussolengo, Italy). After a latency of 24 hours the tibia was lengthened 0.375mm every 12 hours for 15 days, producing an 11.25 mm distraction. The fixator was then left in situ for 27 days to allow the regenerate to consolidate. Pamidronate 3.0 mg/kg diluted to 30mg/100ml was administered as a single intraoperative infusion over two hours to 10 of the rabbits; 10 control animals were given saline infusions. Buprenorphine 0.05 mg/kg was administered at the end of surgery and again 12 hours post operatively. The animals were supplied with rabbit pellet and water ad libitum. At 42 days the rabbits were sacrificed with IV Lethobarb 150mg/kg.

Radiographic and Bone Mineral Density Analysis

Both hind limbs were disarticulated through the knee and the soft tissues left intact. The limbs were oriented in standard AP and lateral projections and plain radiographs taken with a Siemens Multix H/UPH configuration using digital luminescent cassettes with a 50 kV and 4 mA

exposure with a tube to film distance of 1.1 meters. The distance between pin sites was measured from each dissected specimen so that each radiograph could be re-scaled appropriately for measurements of regenerate length.

Bone mineral density (BMD) measurements were made using a total body dual energy x-ray densitometer (LUNAR DPX, Radiation Corps, Madison, Wisconsin). DXA has been used in this context in previous reports. BMD scans were performed with the tibia oriented in the antero-posterior (AP) and lateral projections, using software specifically designed for measuring small animals (LUNAR DPX, Small Animal Software, 1.0c LUNAR, Radiation Corps, Madison, Wisconsin). The "HiRes <0.5kg Slow" scan mode was used ("Fine" collimation, sample size of 0.6 x 1.2 mm, and sample interval of 1/16 seconds). To calculate CV's of the machine, thirty scans were performed on a rabbit forelimb over the duration of the study period. CV's of the BMD, measured by positioning three boxes on each scan, were 3.6%, 4.5% and 5.7% respectively (from proximal to distal).

Regional BMD measurements were obtained by placing "regions of interest" (ROI's) 9.6 mm high on the scan images. For each lengthened tibia, one ROI was positioned in the regenerate, one proximal to it and one distal to it. In the non-operated tibia, two ROI's were placed so that they matched the distal and proximal ROI of the lengthened tibia (ie the same distance from the bone ends). A total of three measurements were thus generated for each lengthened tibia and two measurements for each non-operated tibia, for each projection. BMD values were expressed as g/cm^2 and group data reported as mean and standard deviation. Lengthened and non-operated tibia samples were compared using paired t-tests; non-paired tests were used to compare between groups.

Histological Analysis

The histological analysis was performed in a blinded fashion by two pathologists who were observers. Five pairs of tibiae from the Pamidronate group and five controls were excised sub-periosteally and fixed in 10% buffered formalin. Each bone was transversely sectioned into proximal, regenerate and distal bone segments prior to decalcification in standard EDTA solution over 48 hours. Each segment was then longitudinally sliced and half embedded in paraffin blocks yielding six blocks per rabbit. Sections for microscopy were cut at 5 microns and stained with haematoxylin and

eosin. Both pathologists examined all the sections and made a consensus assessment of the amount of new bone formation, cortical thickness, extent of remodelling, and bone formation around the pin sites. As cortical thickness would be expected to alter depending on the plane of sectioning, it was not measured precisely. The osteoclast activity in the regenerate was expressed as number per high-powered field (Olympus, BH2, 40x objective) The degree of osteoblastic rimming was also assessed.

Results:

Exclusions

There were 3 postoperative complications necessitating exclusion from DXA analysis: one tibial fracture noted on day 1 near the distal pin sites (pamidronate group); one femoral fracture on day 23 requiring euthanasia (pamidronate group); and one common peroneal nerve palsy (control).

Control Rabbit Model

Reliable bone formation occurred in the distraction gap. All tibiae were clinically and radiographically united at day 42. BMD values from the AP scan for the lengthened and non-operated limbs are shown in figure 2. There was a significant reduction in BMD in both the proximal and distal segments surrounding the lengthening compared with the matched sites in the non-operated limb ($p<0.02$). Similar significant differences were present on the lateral scans.

Pamidronate Group

The reduction in BMD in the proximal and distal segments was not found in the pamidronate group, with no significant difference in BMD between the bone of the operated and non-operated limbs at six weeks ($p=0.332$ proximal, $p=0.256$ distal) (Figure 3). The same effect was seen on the lateral scans.

Figure 4 compares the BMD from AP scans of the operated limbs for rabbits given pamidronate versus controls. The BMD of the proximal and distal bone surrounding the regenerate has increased by a mean of 40% and 39% respectively ($p<0.01$). The BMD in the regenerate was increased by a mean of 43% over the control rabbit tibiae ($p=0.017$). Lateral scans also confirmed the above significant differences.

The BMD for the non-operated limbs of control and pamidronate groups was not significantly different. These results are compared in Table 1. There was an increase in the mean regenerate area of 22% in the

pamidronate group ($p<0.05$).

Histology

Of the ten specimens collected, nine were examined histologically. The rabbit culled on day 23 was not included in the analysis. The tibiae from the pamidronate group took 24-48 hours longer to decalcify than the control specimens. The pamidronate group demonstrated increased regenerate formation with prominent osteoblastic rimming and decreased numbers of osteoclasts (2.4 / HPF v 3.4 / HPF in controls) with less evidence of bone remodelling (Fig 5). There was also increased endosteal bone formation in the bone cortex adjacent to the regenerate, producing an increase in cortical thickness (Fig. 5). There was a marked increase in bone formation around the pin sites in the pamidronate group. There was a degree of variability in these factors within the pamidronate group, as with the variability seen in BMD values.

Discussion:

In this experiment, a single dose of 3 mg/kg of pamidronate was given to the rabbits at the beginning of the lengthening to minimise negative effects on bone remodelling. This strategy abolished the osteoporosis seen in controls and had a markedly positive effect on osteoblast activity and bone mineral accretion in the regenerate. Osteoclast number and activity remained reduced at day 42. As the pamidronate was given at the time of surgery, the marked increase in regenerate formation and mineralisation is most interesting. Either pamidronate from the surrounding bone leeched out into the regenerate bone to exert a local effect, or the increase in regenerate was due to an anabolic effect secondary to changes in PTH and 1,25-$(OH)_2$ vitamin D. Further research is required to evaluate these hypotheses.

It was hypothesised that a pulsatile pre-dosage regimen would be desirable when coincident with surgical intervention. As pamidronate has a strong affinity for bone mineral, it is possible to load the skeleton with a pulsatile dose that will exert a positive effect for three to six months (Glorieux et al, "Cyclic administration of pamidronate in children with severe osteogenesis imperfecta" *N. Engl. J Med* 1998; 339: 947-52). Li et al (*supra*) set out to determine if patients receiving continuing bisphosphonate therapy should have this treatment withdrawn in the event of a fracture. They showed increased callus formation in rat femoral fractures pre-treated with incadronate. Continuation of therapy for sixteen weeks after fracture

increased callus formation even further, but the new bone did not remodel to form a cortical shell as in the pre-treated group. They concluded that the therapy should be ceased to allow remodelling, but that more study was needed.

Apart from negative effects on remodelling, continuous dosing with bisphosphonates may not be desirable in distraction osteogenesis because of a detrimental effect on longitudinal growth. In a study using daily subcutaneous alendronate, normal growing mice showed diminution in leg length, as well as decreased ductility after treatment (Raggio et al, "Alendronate reduces fractures without increasing bone strength in a growing mouse model of osteogenesis imperfecta" *Proceedings of Paediatric Orthopaedic Society of North America*, May, 1999). In the experiment, no significant change in leg length was noted, although there was a suggestion of growth inhibition – the non-operated limbs in the control group were longer than those of the pamidronate group by 1.4mm. There were no limb measurements taken at the time of operation, so this difference could not be attributed to growth inhibition with absolute certainty. However, growth inhibition may well have been minimised by the one dose regimen. The study of Li et al (*supra*) did not comment on longitudinal growth, but the comparison radiographs indicate that the continually treated rat femora were shorter than those of the pre-treated and control groups.

The histologic findings in this study suggest an increase in bone formation, as well as a decrease in bone resorption following the administration of pamidronate. This was found particularly in the endosteal region of the bone surrounding the lengthening as well as in the regenerate, and was more obvious in the operated leg. Other studies have recently suggested that bisphosphonates influence cells of osteoblastic lineage in a fashion distinct from their inhibitory effects on osteoclasts (Giuliani et al, "Bisphosphonates stimulate formation of osteoblast precursors and mineralized nodules in murine and human bone marrow cultures in vitro and promote early osteoblastogenesis in young and aged mice in vivo" *Bone* 1998, May; 22(5):455-61).

As pamidronate increased the bone forming capacity of the regenerate, it is possible that it's use may increase the risk of premature consolidation. The length of the regenerate was decreased by a mean of 0.8 mm (8%) in the rabbits given pamidronate. While this small amount is not alarming, the

difference did reach statistical significance, such that careful observation for this possibility would be required if pamidronate were used clinically.

One rabbit given pamidronate sustained a femoral fracture in their operated limb on day 23. This may have been a random event, however it is possible that the bone was made brittle by the pamidronate.

The toxicological effects of bisphosphonates in children are yet to have been fully evaluated. The dose of 3 mg/kg for this experiment was chosen because that is the dose commonly given to children with osteogenesis imperfecta (Glorieux et al, *supra*).

The large increase in BMD throughout the lengthened limb that were obtained following a single dose of pamidronate may have a positive therapeutic effect in children undergoing limb lengthening. The fact that pamidronate increased the amount of regenerate that formed provides a promising prospect worth continuing evaluation. Further investigations into the mechanical properties of the bone after pamidronate treatment, refinement of the dosage regimen and examination of possible toxicological effects are required prior to a clinical trial.

Table I

Comparison of Mean Data between Pamidronate and Control Groups Standard Deviation, upper and lower 95% Confidence Limits in Parentheses

Variable	*Pamidronate*	*Control*	*Difference*	*95% CI*	*p value*
Length of lengthened limb (mm)	106.0 (3.4)	107.6 (4.5)	-1.57	(-5.76, 2.63)	0.44
Length of non-operated limb (mm)	97.4 (2.0)	98.8 (2.2)	-1.45	(-3.59, 0.74)	0.18
Weight of lengthened tibia (g)	10.9 (0.6)	8.7 (0.9)	2.2	(1.37, 3.03)	<0.001
Weight of non-operated tibia (g)	9.3 (0.5)	8.1 (0.4)	1.19	(0.71, 1.67)	<0.001
Regenerate length (mm)	9.6 (0.6)	10.4 (0.7)	-0.75	(-1.45,-0.04)	0.04
Regenerate area (cm^2)	0.83 (0.09)	0.68 (0.13)	0.15	(0.03, 0.27)	0.017
AP BMD proximal to regenerate (g/cm^2)	0.51 (0.07)	0.36 (0.09)	0.14	(0.06, 0.23)	0.004
AP BMD in regenerate (g/cm^2)	0.47 (0.11)	0.33 (0.11)	0.14	(0.03, 0.25)	0.017
AP BMD distal to regenerate (g/cm^2)	0.48 (0.10)	0.34 (0.08)	0.14	(0.04, 0.23)	0.007
AP BMD in proximal non-operated limb (g/cm^2)	0.48 (0.05)	0.44 (0.03)	0.04	(0.00, 0.09)	0.053
AP BMD in distal non-operated limb (g/cm^2)	0.44 (0.04)	0.42 (0.02)	0.02	(-0.02, 0.05)	0.31

Summary of Findings:

The effect of a single 3 mg / kg dose of pamidronate (Novartis) on Bone Mineral Density (BMD) was examined in a distraction osteogenesis model in immature rabbits.

Seventeen rabbits (9 control, 8 given pamidronate) were examined by Dual X-ray Absorbtiometry (DXA). There was a significant increase in BMD in the pamidronate group over controls. The mean areal BMD (g/cm^2) in the bone proximal and distal to the regenerate bone was increased by 40% and 39% respectively compared to controls ($p<0.05$). The BMD of the regenerate bone was increased by a mean of 43% over controls ($p<0.05$). There was also a 22% increase in mean area of regenerate formed in the pamidronate group ($p<0.05$).

Histological analysis of nine rabbits (5 control, 4 pamidronate) revealed an increase in osteoblastic rimming and mineralisation of the regenerate in the pamidronate rabbit tibiae. There was also increased bone formation around the pin sites and an increase in the cortical width of the bone adjacent to the regenerate in the rabbits given pamidronate.

Pamidronate had a markedly positive effect in this limb-lengthening model. Not only did it reduce the disuse osteoporosis normally associated with lengthening using an external fixator, it also increased the amount and density of the regenerate bone. Further study to examine the mechanical properties of the regenerate after administration of pamidronate is required.

Example 2: Pamidronate in Distraction Osteogenesis (dose 1mg/kg)

Methods:

Experimental design

Twenty eight-week-old male NZW rabbits underwent tibial lengthening. After premedication with IM Ketamine 15 mg/kg and Xylazine 4mg/kg, anaesthesia was administered with Halothane 2%, and Oxygen 1 l/min. An open mid-tibial drill hole osteotomy was performed on each rabbit and an Orthofix M-100 fixator was applied using four Orthofix 3 mm half pins (Orthofix, Bussolengo, Italy). After a latency of 24 hours the tibia was lengthened 0.375mm every 12 hours for 15 days, producing an 11.25 mm distraction. The fixator was then left in situ for 27 days to allow the regenerate to consolidate. Pamidronate 1.0 mg/kg diluted to 30mg/100 ml was administered as a single intraoperative infusion over two hours to 10 of the rabbits: 10 control animals were given saline infusions. Buprenorphine

0.05 mg/kg was administered at the end of surgery and again 12 hours post operatively. The animals were supplied with rabbit pellet and water ad libitum. At 42 days the rabbits were sacrificed with IV Lethobarb 150mg/kg.

Radiographic and Bone Mineral Density Analysis

Both hind limbs were disarticulated through the knee and the soft tissues left intact. The limbs were oriented in standard AP and lateral projections and plain radiographs taken with a Siemens Multix H/UPH configuration using digital luminescent cassettes with a 50 kV and 4 mA exposure with a tube to film distance of 1.1 meters. The distance between pin sites was measured from each dissected specimen so that each radiograph could be re-scaled appropriately for measurements of regenerate length.

The disarticulated bones were stripped of all soft tissue and analysed using a Stratec XCT-960A pQCT scanner and analysis software (Stratec Medizintechnik Gmbh, Pforzheim, Germany). Two millimetre slices were obtained, 15 slices in the right (lengthened) tibiae and 10 in the non-operated tibiae. Five slices were thus obtained in the regenerate, proximal and distal bone, and corresponding areas to the proximal and distal bone in the non-operated limb. Quantitative CT is the noninvasive method with the strongest predictive power for the mechanical strength of newly formed bone (Harp et al., 1994). The software allowed analysis and generation of data on bone mineral density as mg/cm^3, bone mineral content (mg) and cross sectional area (mm^2). Data was also generated for mechanical analysis, namely moment of inertia (mm^4), and maximum y co-ordinate (vertical distance from the neutral bending plane in mm).

Strain was calculated for each specimen by formula 1:

$$\text{Strain} = \frac{12Dy}{L^2}$$

D= deflection

y= vertical distance from centre of mass

L= span length

Stress was calculated at 2 mm intervals along the central section of specimen from formula 2:

$$\text{Stress} = \frac{My}{I}$$

M= moment at x

$$= \frac{load}{2}x - \frac{load}{2}(x - L/4)$$

y= vertical distance from centre of mass

I= second moment of inertia

x = distance along bone from left roller

The Young's modulus of elasticity was calculated as the slope of the linear portion of the stress/strain curve for each "slice" or 2 mm interval.

The mean Young's modulus values for the control and treated groups at both the central "slice" and for averaged data over the central 1 cm of regenerate section, were compared using an unpaired two tailed t-test.

The area under the stress strain curves for the central "slice" was calculated for the distracted tibiae in both control and pamidronate groups. These were again compared with an unpaired two-tailed t test.

Results:

Reliable bone formation occurred in the distraction gap. All tibiae were clinically and radiographically united at day 42. There was a significant increase in bone mineral density proximal and distal to the regenerate in the pamidronate group (Table II). There were also significant increases in bone mineral content. There was a 13% increase in bone area, although this was not statistically significant (p=0.2).

The lengthened tibiae in the pamidronate group were 32% stronger for peak load (p=0.004) (Fig 6). The peak load in the lengthened tibiae in the pamidronate group was the same as that for the non-operated control tibiae. Young's modulus was not significantly different between operated groups, and was reduced to only about 30% of the value of the in tact tibiae (Fig 7).

Discussion:

In this experiment, a single dose of 1.0 mg/kg given to the rabbits at the beginning of the lengthening produced significant improvement in peak load at six weeks measured by four point bending. As in our previous experiment, osteoporosis surrounding the lengthening was reduced. Although not statistically significant, there was an increase in callus area, but not as marked as had occurred in specimens from the group in the previous experiment treated with 3.0 mg/kg of pamidronate.

The increase in peak load is likely to be due to both increased regenerate volume and increased mineral content at six weeks. The fact that modulus of elasticity remained unchanged with pamidronate indicates that

the bone may be no more mature than in controls. Combining bisphosphonate treatment and mechanical stimulation may prove to be beneficial; the hypothesis being that the bisphosphonate will increase early osteoblastic proliferation and increase bone mineral content, while the mechanical stimulation would accelerate callus maturation.

TABLE II

Comparison of Data between Control and Pamidronate Groups						
	Control		Pamidronate			
	Mean	SD	Mean	SD	% increase	P value
TOT BMC Prox	27.98	6.68	34.52	6.27	23%	0.03
TOT BMC Regen	26.13	10.70	30.47	6.67	17%	NS
TOT BMC Dist	28.04	4.65	34.47	4.34	23%	0.004
Total BMD Prox	585.53	69.61	650.73	47.06	11%	0.03
Total BMD Regen	530.06	77.61	575.03	62.12	8%	NS
Total BMD Dist	650.40	79.40	739.37	56.48	14%	0.01
Area Prox	52.97	7.94	48.26	7.41	10%	NS
Area Regen	53.13	9.71	46.90	11.91	13%	NS
Area Dist	46.85	5.73	43.37	6.54	8%	NS

Example 3: Zoledronate in Distraction Osteogenesis (dose 0.1mg/kg)

Methods

Experimental Design

Twenty-four eight-week-old male NZW rabbits underwent tibial lengthening. Similar rabbit models have been reported. After premedication with IM Ketamine 15 mg/kg and Xylazine 4mg/kg, anaesthesia was administered with Halothane 2%, and Oxygen 1 l/min. After preparation of the right lower extremity we performed an open mid-tibial drill hole osteotomy and applied an Orthofix M-100 fixator using four Orthofix 3 mm half pins (Orthofix, Bussolengo, Italy). The left lower extremity was left in tact. After a latency of 24 hours the tibia was lengthened 0.375mm every 12 hours for 14 days, producing a total of 10.5 mm of distraction. The fixator was then left in situ for 28 days to allow the regenerate to consolidate.

The animals were randomised such that eight animals were operated on and given saline-only infusions (controls), eight animals were given 0.1 mg/kg zoledronate over 20 minutes at the time of surgery (single dose zoledronate), and a further eight animals were given a second dose of zoledronate 0.1 mg/kg on day 14 (re-dosed zoledronate).

Buprenorphine 0.05 mg/kg was administered at the end of surgery and again 12 hours post operatively to all animals. The animals were supplied

with rabbit pellet and water ad libitum. At 42 days the rabbits were sacrificed with IV Lethobarb 150mg/kg.

Radiographic and Bone Mineral Density Analysis

Bone mineral content (BMC) and density (BMD) measurements were made at two, four and six weeks using a total body dual energy x-ray densitometer (LUNAR DPX, Radiation Corps, Madison, Wisconsin). DXA scans were performed with the tibia oriented in a jig in the antero-posterior (AP) projection, using software specifically designed for measuring small animals (LUNAR DPX, Small Animal Software, 1.0c LUNAR, Radiation Corps, Madison, Wisconsin). The "HiRes <0.5kg Slow" scan mode was used ("Fine" collimation, sample size of 0.6 x 1.2 mm, and sample interval of 1/16 seconds).

Regional BMC and BMD measurements were obtained by placing "regions of interest" (ROI's) 9.6 mm high on the scan images. For each lengthened tibia, one ROI was positioned in the regenerate, one proximal to it and one distal to it. In the non-operated tibia, two ROI's were placed so that they matched the distal and proximal ROI of the lengthened tibia (ie the same distance from the bone ends). A total of three measurements are thus generated for each lengthened tibia and two measurements for each non-operated tibia. BMC values were expressed in grams (g) and BMD values expressed as g/cm^2 and group data reported as mean, standard deviation and 95% confidence intervals.

After culling, both hind limbs were disarticulated through the knee and the soft tissues left intact. The limbs were oriented in standard AP and lateral projections and plain radiographs taken with a Siemens Multix H/UPH configuration using digital luminescent cassettes with a 50 kV and 4 mA exposure with a tube to film distance of 1.1 meters. A calibrated marker on the film allowed the image to be re-scaled appropriately for measurements of length in millimetres (mm).

To expand the analysis at six weeks the disarticulated bones were then stripped of all soft tissues and analysed using a Stratec XCT-960A pQCT scanner and analysis software (Stratec Medizintechnik Gmbh, Pforzheim, Germany). Two millimetre slices were obtained, 15 slices in the right (lengthened) tibiae and 10 in the non-operated tibiae. Five slices were thus obtained in the regenerate, proximal and distal bone, and corresponding areas to the proximal and distal bone in the non-operated limb. The software

allowed analysis and generation of data on bone mineral density as g/cm^3, bone mineral content (g) and cross sectional area (mm^2). Data was also generated for mechanical analysis, namely moment of inertia (mm^4), and maximum y co-ordinate (vertical distance from the neutral bending plane in mm).

Results

Exclusions

One rabbit in the single dose zoledronate group died suddenly of a gastrointestinal illness 9 days after surgery. One rabbit in the re-dosed group had failure of distraction and premature consolidation and was excluded. This left 8 rabbits in the control group and seven rabbits in each of the zoledronate groups.

Bone Mineral Content (BMC)

The BMC as measured by DXA was similar at two weeks in all groups (Fig. 8). There was a rapid increase in mineralisation of all three regions of the operated limb between week two and week four in the zoledronate treated animals. This was significantly different for treated groups over controls in all regions except for single dose animals in the distal segment (t test $p<0.01$). There was a fall off in BMC between weeks four and six in all regions. This was most marked in control animals, much reduced in the single dose group and minimal in the double dose group, such that both treated groups had significantly increased BMC at six weeks over controls. The difference between single dose and redosed animals for BMC was significant at six weeks in the proximal and distal segments, but not in the regenerate ($p<0.01$).

The velocity of bone mineral accrual in the regenerate was significantly higher between weeks 2 and 4 in the treated animals (Fig. 9, $p<0.01$). Between weeks 4 and 6, when all groups shed some bone mineral, this was least so in the redosed zoledronate group ($p<0.05$ v control, NS v single dose).

At six weeks the BMC as measured by QCT was increased significantly in all regions in the zoledronate treated animals ($p<0.01$, ANOVA) (Fig. 10). The effect was enhanced in the double dose group in a dose-related fashion. Post-hoc t-tests revealed that the differences between single dose versus control and double dose versus single dose were both significant ($p<0.05$). There was no significant change in the BMC in the non-operated tibiae.

Bone Mineral Density

The control group BMD in the regenerate as measured by DXA increased between weeks 2 and 4 but dropped off again to the 2-week value (Fig. 11). The BMD dropped off progressively in the proximal and distal regions, the expected effect of stress shielding (Fig. 11). In contradistinction, the BMD of the regenerate in the zoledronate treated animals increased more rapidly, and was largely maintained. The zoledronate served to protect the proximal and distal regions from the effects of stress shielding such that the BMD was maintained above two-week levels at six weeks. Both zoledronate treated groups had areal BMD values significantly different from controls at both 4 and 6 weeks by DXA, but the single dosed and redosed groups were never significantly different from each other for areal BMD.

At six weeks the volumetric BMD as measured by QCT was increased significantly in all regions in the zoledronate treated animals ($p<0.01$, ANOVA) (Fig. 12). Post-hoc t-tests revealed that the differences between single dose versus control ($p<0.01$), but no difference between re-dosed versus single dose ($p>0.05$). There was no significant change in the BMC in the non-operated tibiae. The zoledronate treated animals maintained a BMD in all regions similar to that of the non-operated control tibiae whereas the control distracted tibiae showed a significant amount of osteoporosis in all regions.

Length Measurements at six weeks

There was a dose related difference in the lengths of the non-operated tibiae of the rabbits, such that the mean tibial length of the single zoledronate group was reduced by 3% over controls and the re-dosed group reduced by 7% (ANOVA $p<0.01$ Table III). The re-dosed zoledronate group had significantly shorter operated right tibiae as well, but the regenerate lengths were not different between the groups. These data suggest a small but definite dose-related negative effect of zoledronate in longitudinal growth at the physis.

Cross-sectional Area Measurements at six weeks

There was a significant dose-dependant increase in cross sectional area in all regions in zoledronate treated animals as measured by QCT ($p<0.01$, ANOVA). There was no effect at all on the cross sectional area of the non-operated tibiae (Fig. 13). The most marked effect was seen in the regenerate, with increases of 56% in the single dose group and 105% in the double dose

group. but considerable increases in cross sectional area of the adjacent regions was also seen, ranging from 29% to 72%. The median QCT scan for each group in terms of cross sectional area is shown in Figure 14

There were even larger increases in moment of inertia. as this is proportional to r^4 (Fig.14). The moment of inertia for the regenerate was thus increased by 111% in the single dose group and by 213% in the double dose group ($p=0.02$ ANOVA). The difference between single dose zoledronate and controls was significant by post-hoc unpaired t test ($p=0.02$). The further rise in moment in the repeat dose group did not reach significance, as the variability was high ($p=0.3$). Increases in moment of inertia in surrounding regions ranged from 57% to 180%.

Further study to examine the mechanical properties of the regenerate after administration of Zoledronate is required.

Discussion

Although given as a simple IV infusion, the significantly beneficial effects of zoledronate administration documented in this study were target organ specific (Figs 10, 12, 13, 14). A massive increase in both the amount of bone formed and its mineralisation, not only in the regenerate but also in adjacent regions was produced. Meanwhile there was little or no effect on the non-operated tibia. Several possible hypotheses may explain this. Although the pathway or even the cell line most directly involved have yet to be defined, zoledronate seems to produce an inability of the bone to sense its mechanical environment. Unlike control tibiae, in which the BMD progressively dropped, bone mineral was not shed in the bone surrounding the osteotomy and distraction. Meanwhile new bone formed in a more vigorous fashion than in controls, even though the bone was rigidly held in the fixator.

Another possibility is that osteoclastic inhibition delays remodelling until more than the usual amount of callus is formed. There is also the possibility that the bisphosphonates are acting directly on osteoblasts, perhaps through basic fibroblast growth factor (bFGF). Further study to try and elucidate exactly which growth factors have been stimulated or inhibited by bisphosphonate administration is required.

Of importance is the observation that the BMD was returned to the value of the control non-operated limb and the increases in BMC were largely due to increased amount of new bone of normal density. If the density to

supra-normal levels had simply been increased with no increased amount of bone formed, then the bone would be osteopetrotic, i.e. brittle and with no improvement in strength.

A single IV dose at time the time of surgery lends itself well to the clinical situation when managing skeletal trauma. Zoledronate has a well-documented safety profile in patients with cancer receiving multiple doses (Major P, Lortholary, Hon J *et al.* "Zolendroic acid is superior to pamidronate in the treatment of tumour-induced hypercalcaemia: a pooled analysis." Proc ASCO New Orleans, May 2000 19:209 (Abstract 814).. A single perioperative dose in a well-hydrated trauma patient should be well tolerated, if there is concern about hydration the treatment could be deferred until it was adequate. This would reduce the theoretical possibility of nephrotoxicity or nephrocalcinosis.

In elective situations the administration of IV zoledronate as a 5-20 minute infusion perioperatively should be easily and safely achieved. Further study is required.

If the treating physician considers the response to a single perioperative dose sub-optimal, or the treatment course is unusually prolonged, this study strongly suggests that a further dose will confer additional benefit. However as a single dose provides over 50% increase in new bone formation in this model, a further dose may not be routinely necessary. Likewise in the paediatric population, appropriate consideration must be given to the negative effects on longitudinal growth related to bisphosphonate administration. This study showed a dose related negative effect on longitudinal growth, although this was only 3% in single dose and 7% in re-dosed animals, it suggests that prolonged administration of zoledronate in growing children may be ill-advised.

The increase in cross sectional area is an extremely significant benefit. Regenerate failure has been documented to be largely in bending and fracture failure in long bones occurs in either bending or torsion. In both bending and torsion the moment of inertia is proportional to r^4, or the square of the cross sectional area. The 56% increase in cross sectional area of regenerate thus translates to 111% increase in moment of inertia. The 105% increase in the re-dosed group equates to a 213% increase in moment of inertia. The regenerate strength index was increased also in proportion to the cross sectional area (Table IV).

The effects seen in this experiment are consistent with the above findings relating to pamidronate administration.

As the present model follows the animals for only six weeks, no conclusion regarding remodelling of the new bone formed can be reached. It is intuitive that the cross sectional area will reduce as the bone remodels. Producing such a large amount of callus may allow removal of the fixator before remodelling has occurred. This strategy would allow remodelling to occur in an environment exposed to physiological loading as the effects of the bisphosphonate wore off over time, rather that remodelling in the stress shielded environment of the fixator. Further studies to look at the behaviour of the bone after fixator removal are required.

TABLE III

Tibial and Regenerate Lengths for Control, Zoledronate and Re-dosed Zoledronate Group

		Control	Zoledronate 1	Zoledronate 2
Number		8	7	7
Left Tibia	**Mean**	**98.00**	**95.14***	**91.14***
	SD	1.41	2.48	2.79
Right Tibia	**Mean**	**107.75**	**107.43**	**101.57***
	SD	2.12	4.08	3.21
Regenerate	**Mean**	**10.38**	**11.29**	**10.57**
	SD	1.30	1.50	1.90

* Denotes significantly different by ANOVA and post-hoc unpaired t-test

TABLE IV

Regenerate Strength Index for Control, Zoledronate and Re-dosed Zoledronate Group

	Control	Zoledronate 1	Zoledronate 2
RSI	2.09	3.26	4.49
% above control		56%	114%

It will be appreciated by persons skilled in the art that numerous variations and/or modifications may be made to the invention as shown in the specific embodiments without departing from the spirit or scope of the invention as broadly described. The present embodiments are, therefore, to be considered in all respects as illustrative and not restrictive.

CLAIMS:

1. A drug selected from a group consisting of at least one bisphosphonate when used for promoting bone growth.

2. The drug of claim 1 when used for promoting bone growth at a fracture site.

3. The drug of claim 1 or claim 2 when used for promoting bone growth between a bone and a prosthesis, bone fixation device or any other bone or dental implant.

4. A drug selected from a group consisting of at least one bisphosphonate when used for treating a fracture.

5. The drug of any one of the preceding claims wherein the bisphosphonate is Zoledronate.

6 The drug of any one of claims 1 to 4 wherein the bisphosphonate is Pamidronate.

7. The drug of any one of claims 1 to 4 wherein the drug is a combination of two or more bisphosphonates.

8. Use of a drug selected from the group consisting of at least one bisphosphonate for the manufacture of a medicament for promoting bone growth.

9. Use of the drug of claim 8 for the promotion of bone growth at a fracture site.

10. Use of the drug of claim 8 for the promotion of bone growth between a bone and a prosthesis.

11. Use of a drug selected from a group consisting of at least one bisphosphonate for the manufacture of a medicament for treating a fractured bone.

12. Use of the drug of any one of claims 8 to 11 wherein the drug is Zoledronate.

13. Use of the drug of any one of claims 8 to 11 wherein the drug is Pamidronate.

14. Use of the drug of any one of claims 8 to 11 wherein the drug is a combination of two or more bisphosphonates.

15. A method for treating a fractured bone, the method including administering to a subject with a fractured bone a therapeutically effective amount of a drug selected from a group consisting of at least one bisphosphonate.

16. The method of claim 15 wherein the drug is administered to the subject as a single dose.

17. The method of claim 16 wherein the single dose of drug is administered at an early stage of treatment of the fractured bone.

18. The method of claim 15 wherein the mode of administration is as a perioperative intravenous infusion.

19. The method of claim 15 wherein the mode of administration is oral.

20. The method of claim 15 wherein the mode of administration is transdermal.

21. A method for treating a fractured bone, the method including the steps of:

(a) administering to a subject with a fractured bone a therapeutically effective amount of a drug selected from a group consisting of at least one bisphosphonate; and

(b) providing a vibratory stimulus to the fractured bone.

22. The method of claim 21 wherein the vibratory stimulus is provided by ultrasound stimulation or vibration stimulation.

23. The method of claim 21 or claim 22 wherein the vibratory stimulus includes periodically providing a vibratory stimulus at the resonant frequency of the bone.

24. The method of claim 23 wherein the resonant frequency is calculated as a function of the bone's vibratory response to the vibratory stimulus.

25. The method of any one of claims 21 to 24 wherein the vibratory stimulus is provided at a late stage in the treatment of the fractured bone.

26. The method of any one of claims 21 to 24 wherein the step of providing a vibratory stimulus is concurrent with the step of administering a therapeutically effective amount of the drug.

27. The method of claim 26 wherein the vibratory stimulus is provided and the therapeutically effective amount of the drug is administered at an early stage in the treatment of a fractured bone.

28. The method of claim 24 wherein the vibratory stimulus is provided and the therapeutically effective amount of at least one drug from the class of bisphosphonates is administered at a late stage in the treatment of a fractured bone.

Figure 1

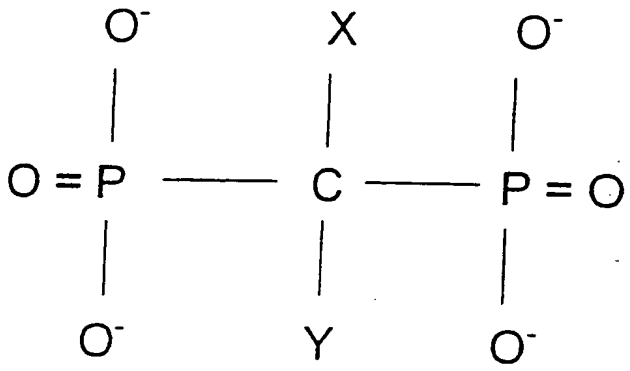


Figure 2

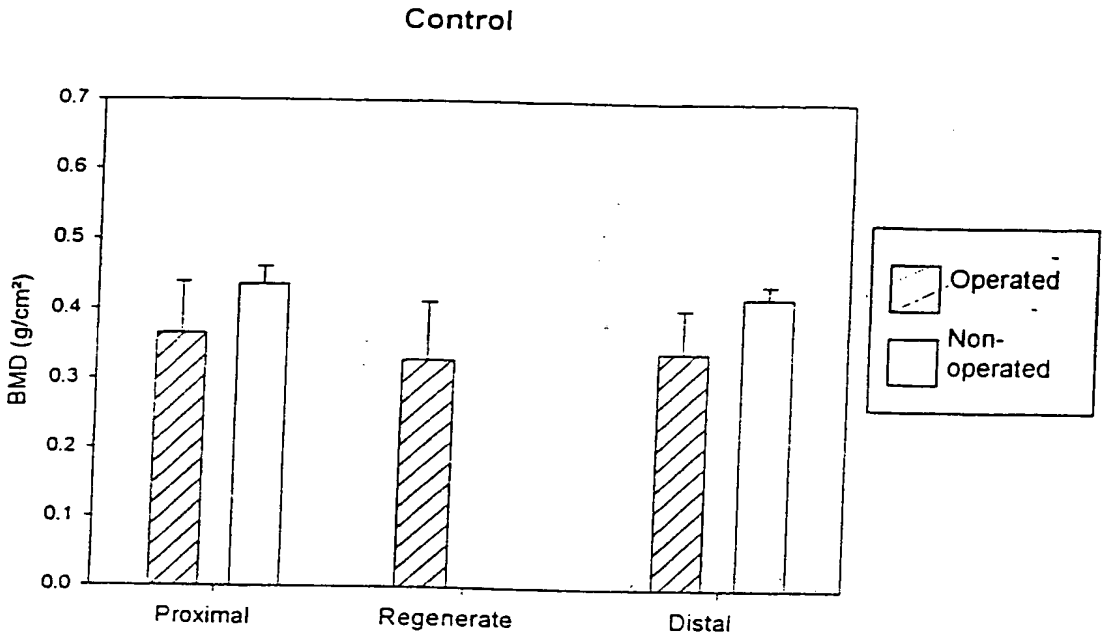

Control
BMD (g/cm²)
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
Proximal
Regenerate
Distal
Operated
Non-operated

Figure 3

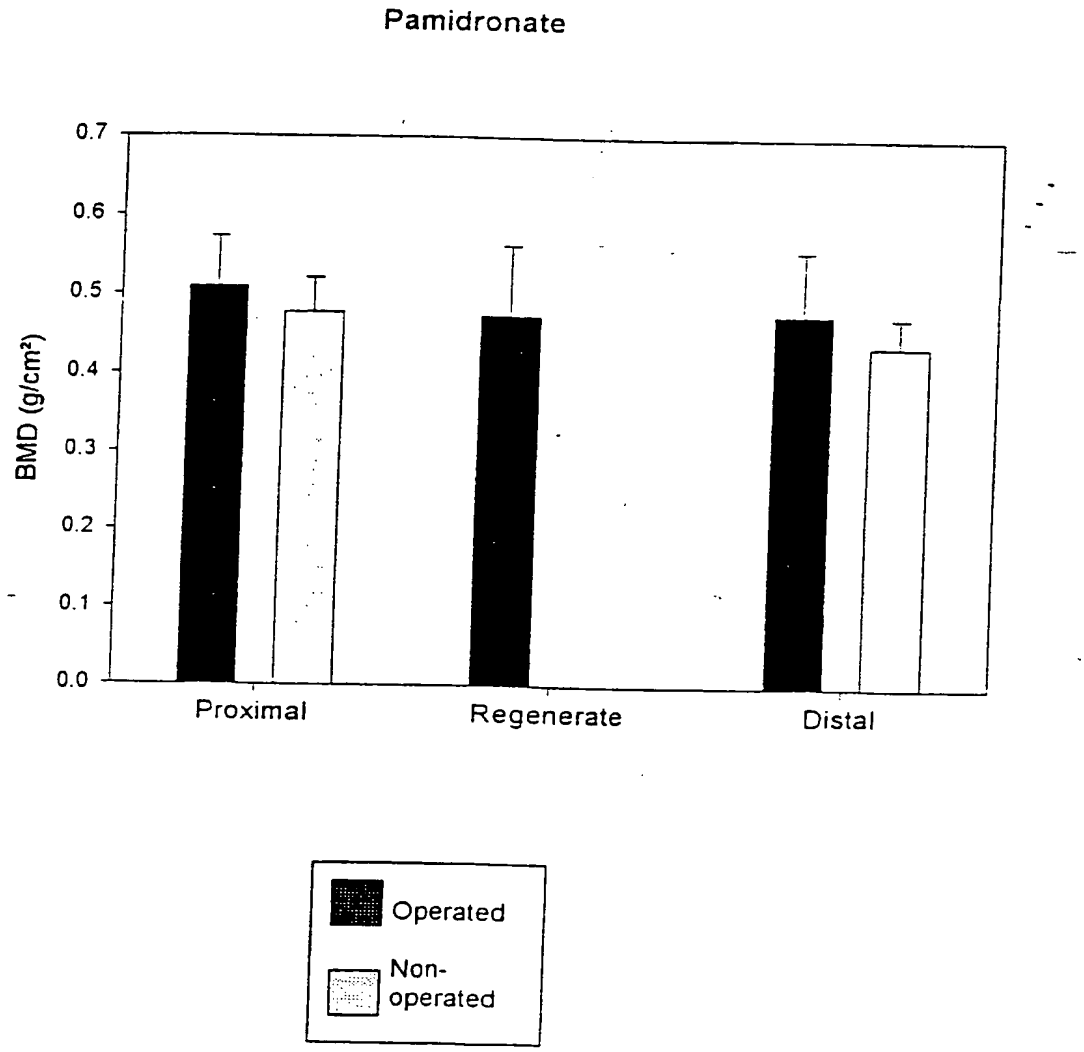


Figure 4

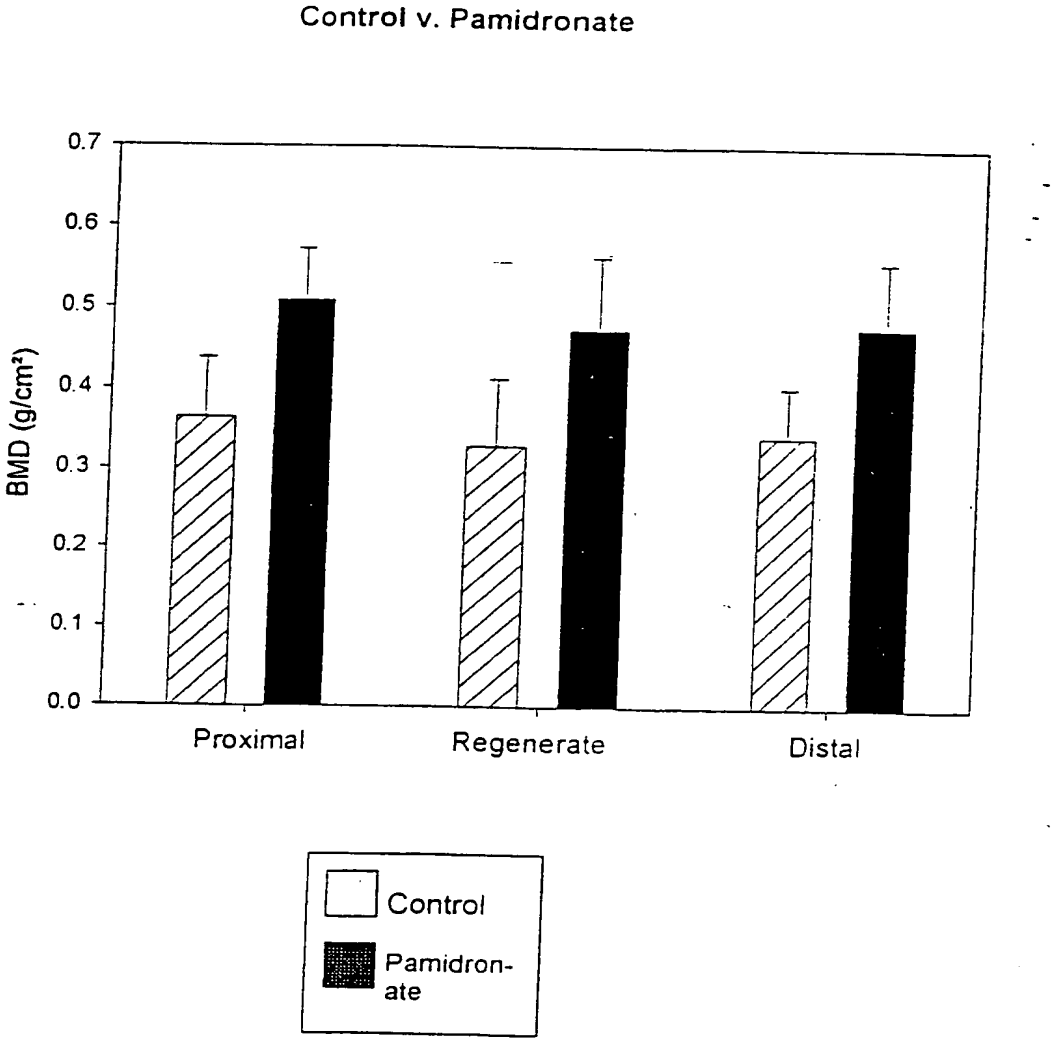

Control v. Pamidronate
BMD (g/cm²)
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
Proximal
Regenerate
Distal
Control
Pamidron-
ate

Figure 5

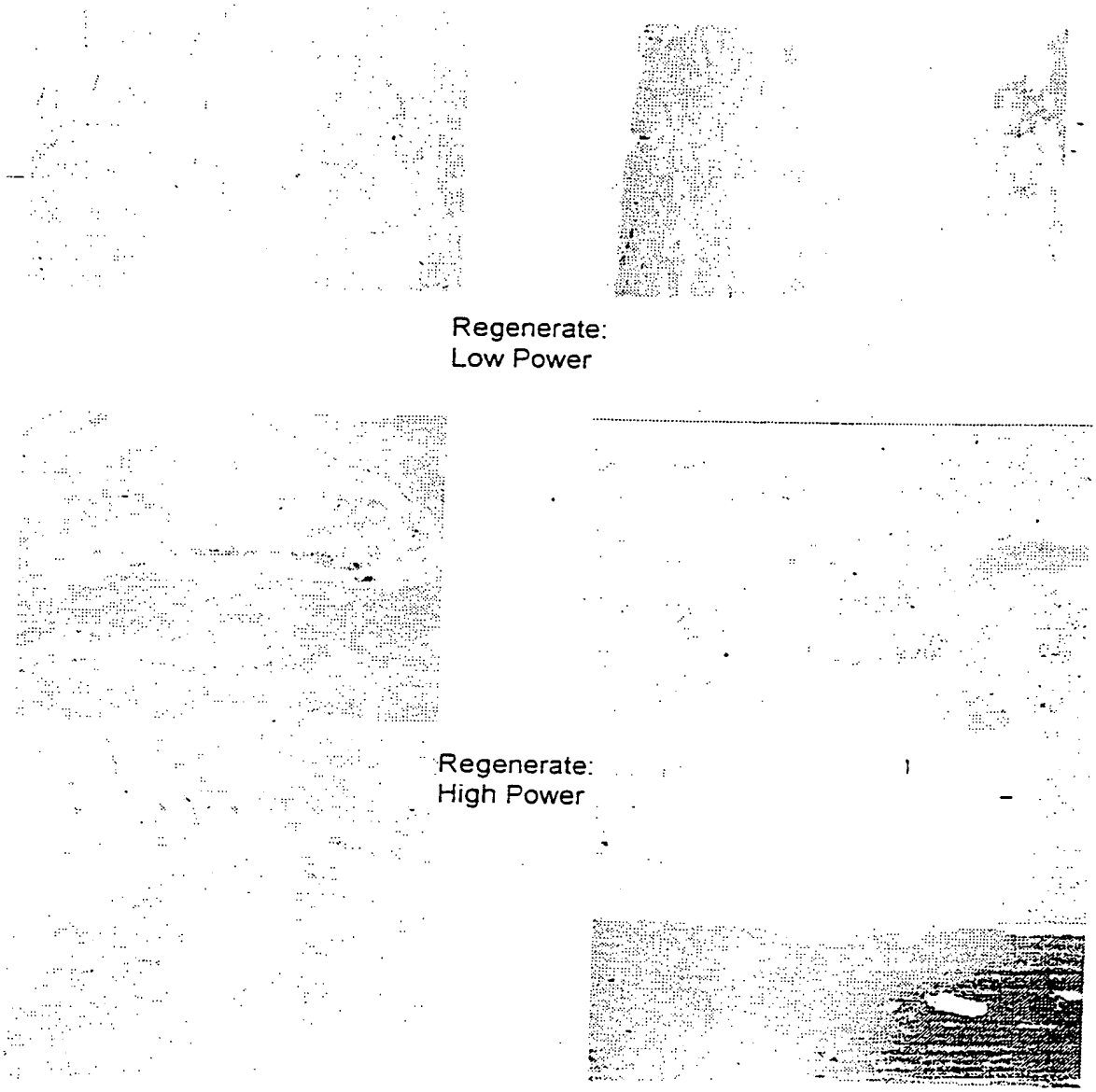

Pamidronate
Control
Regenerate:
Low Power
Regenerate:
High Power
Adjacent
Bone

Figure 6

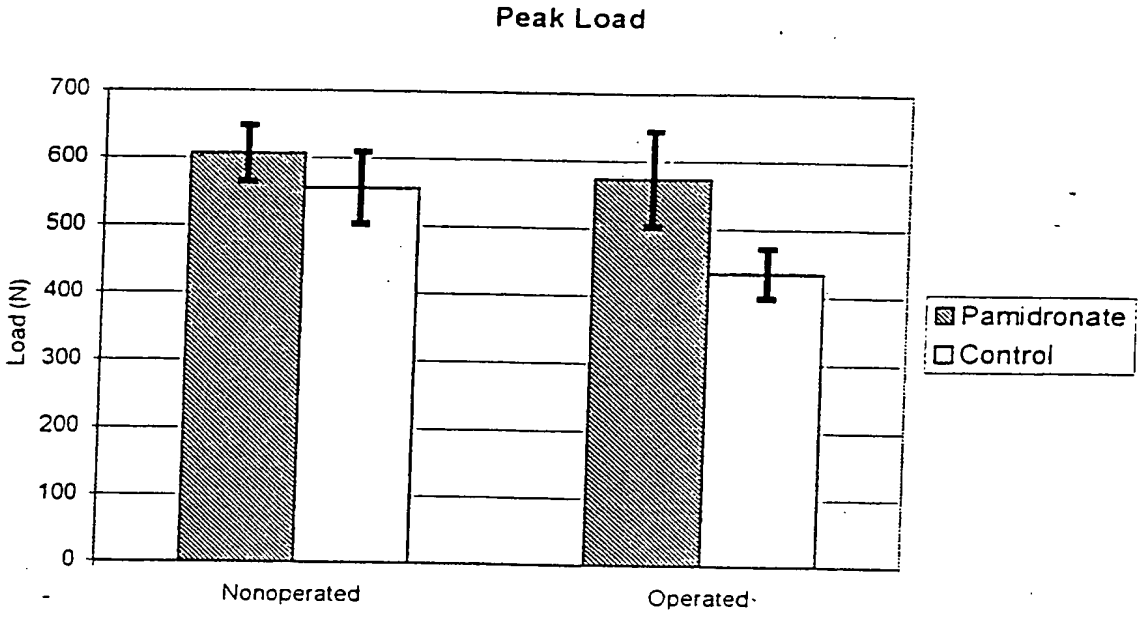

Peak Load
700
600
500
400
300
200
100
0
Load (N)
Nonoperated
Operated
Pamidronate
Control

Figure 7

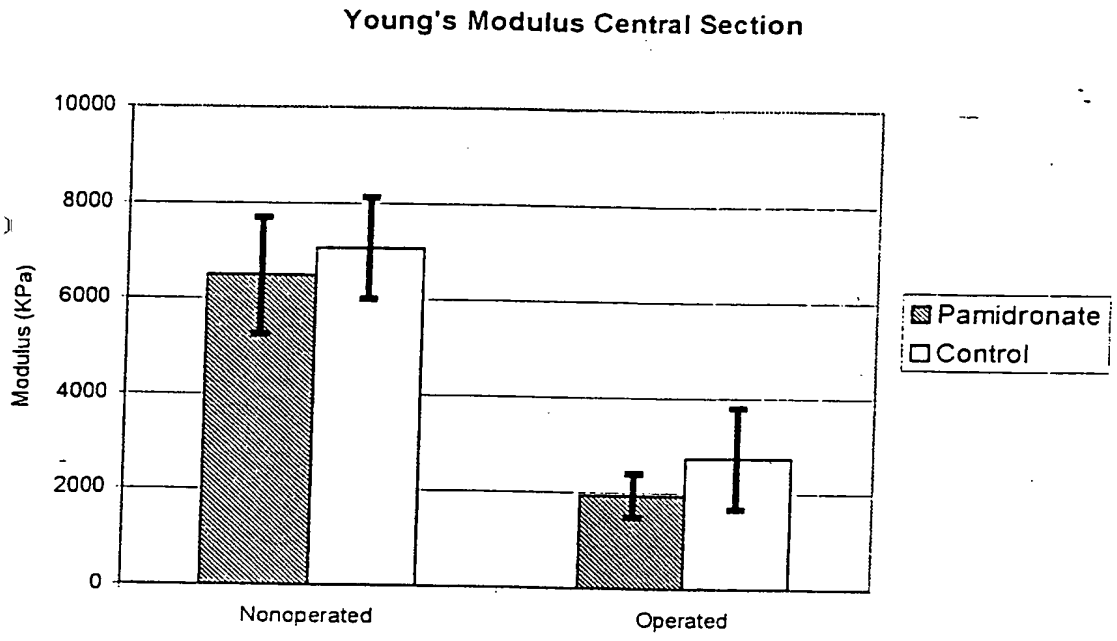

Young's Modulus Central Section
10000
8000
6000
4000
2000
0
Modulus (KPa)
Nonoperated
Operated
Pamidronate
Control

Figure 8A

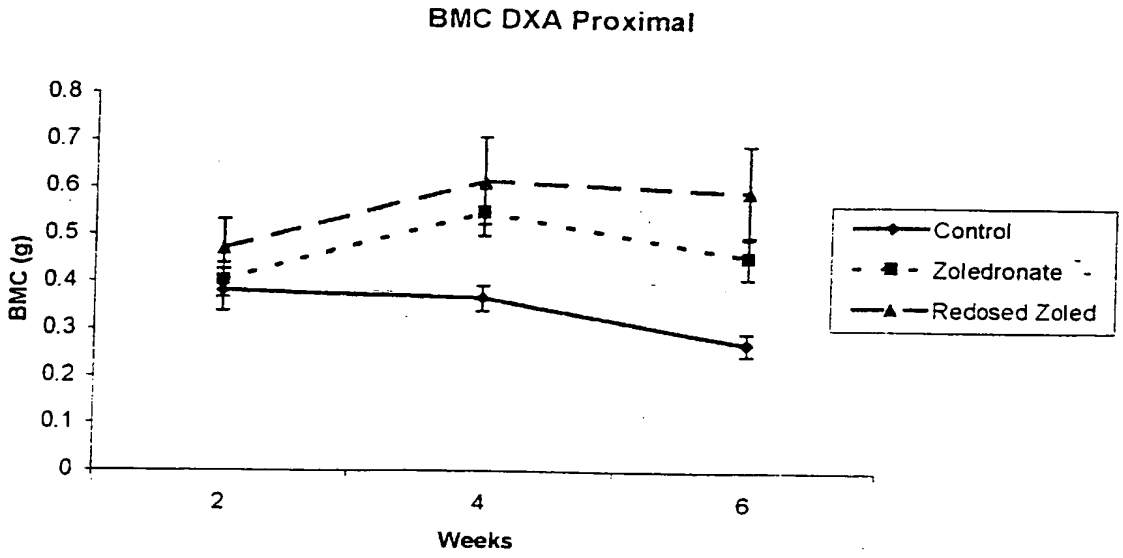

BMC DXA Proximal
BMC (g)
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
2
4
6
Weeks
Control
Zoledronate
Redosed Zoled

Figure 8B

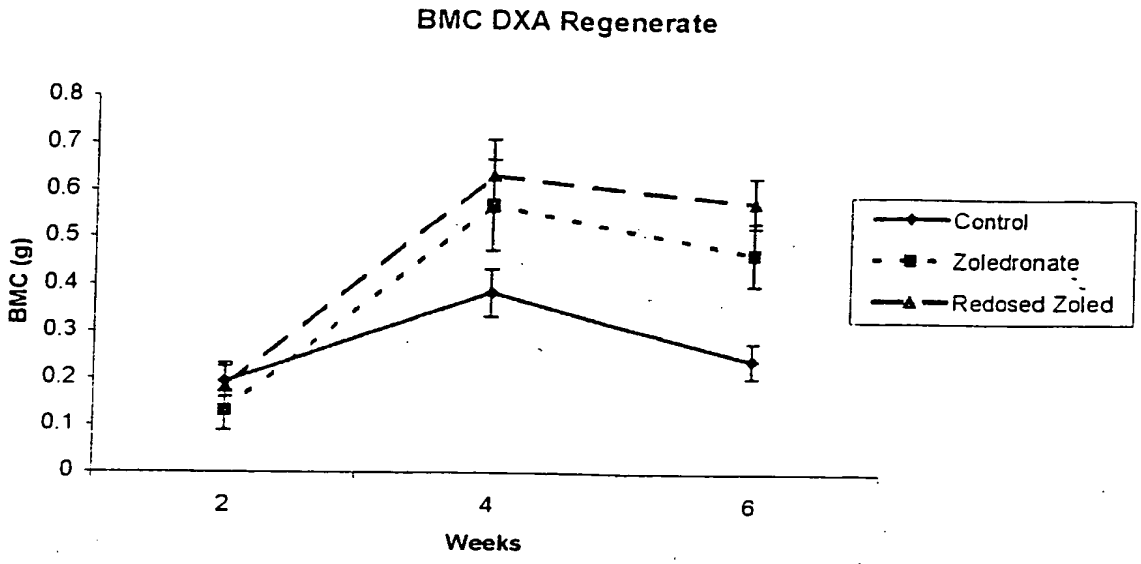

BMC DXA Regenerate
BMC (g)
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
2
4
6
Weeks
Control
Zoledronate
Redosed Zoled

Figure 8C

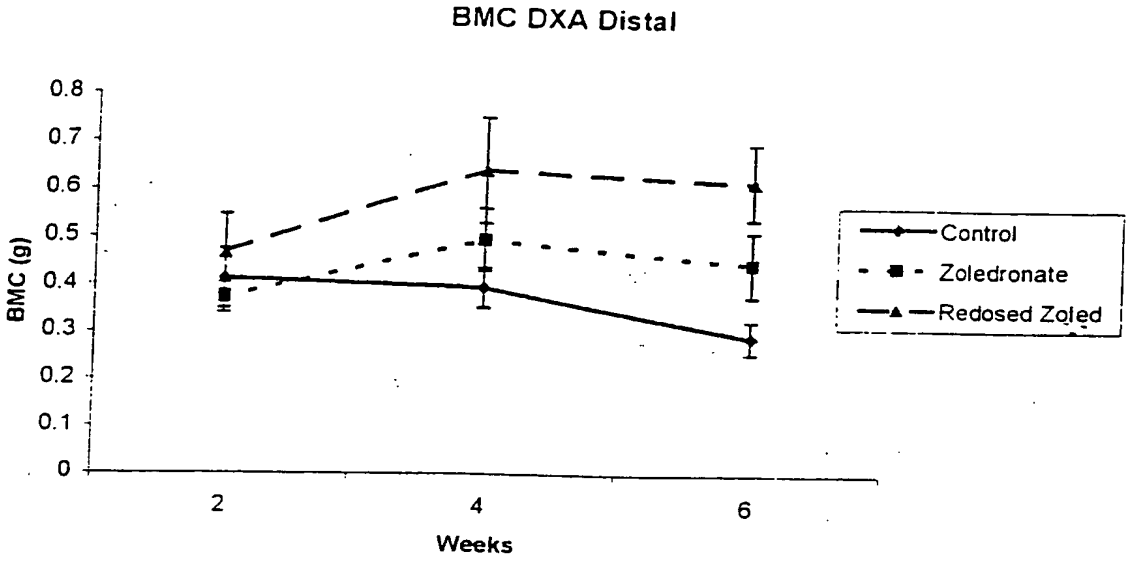

BMC DXA Distal
BMC (g)
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
2
4
6
Weeks
Control
Zoledronate
Redosed Zoled

Figure 9

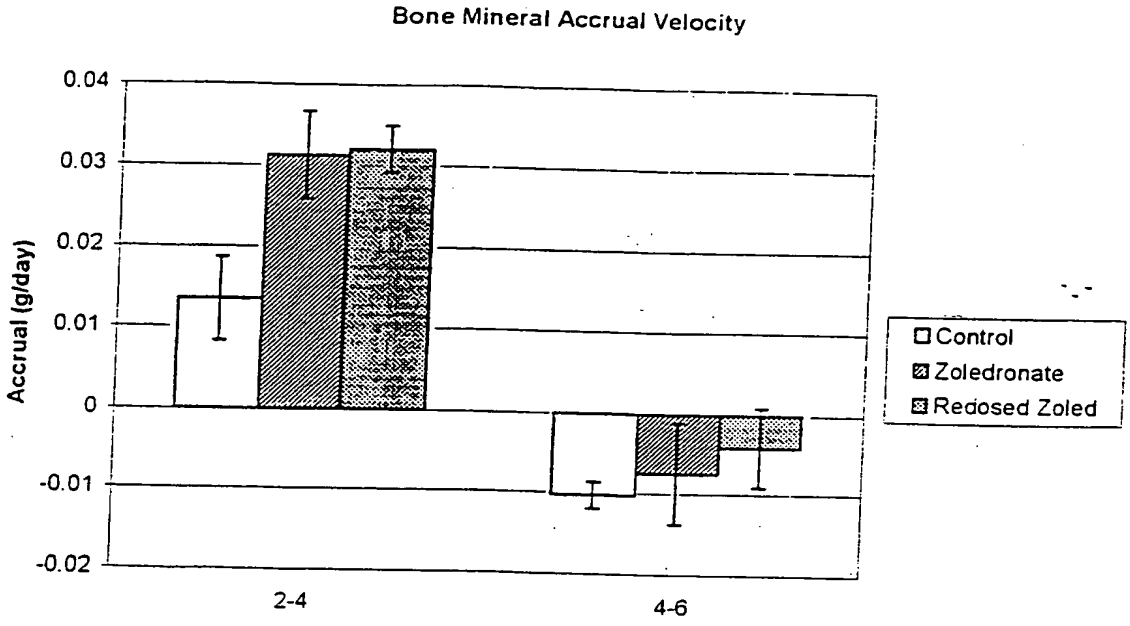

Bone Mineral Accrual Velocity
Accrual (g/day)
0.04
0.03
0.02
0.01
0
-0.01
-0.02
2-4
4-6
Control
Zoledronate
Redosed Zoled

Figure 10

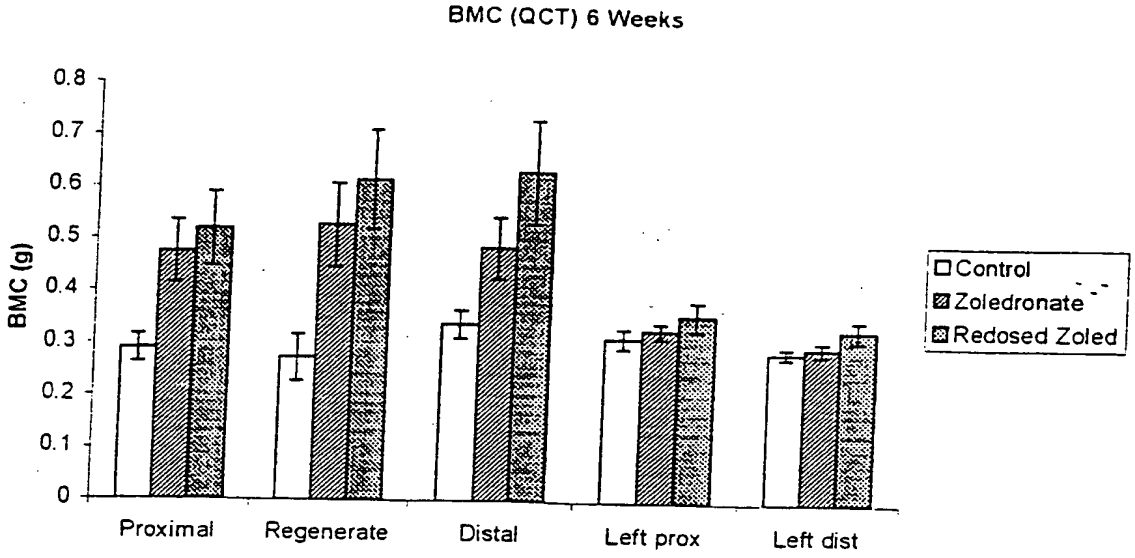

BMC (QCT) 6 Weeks
BMC (g)
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Proximal
Regenerate
Distal
Left prox
Left dist
Control
Zoledronate
Redosed Zoled

Figure 11A

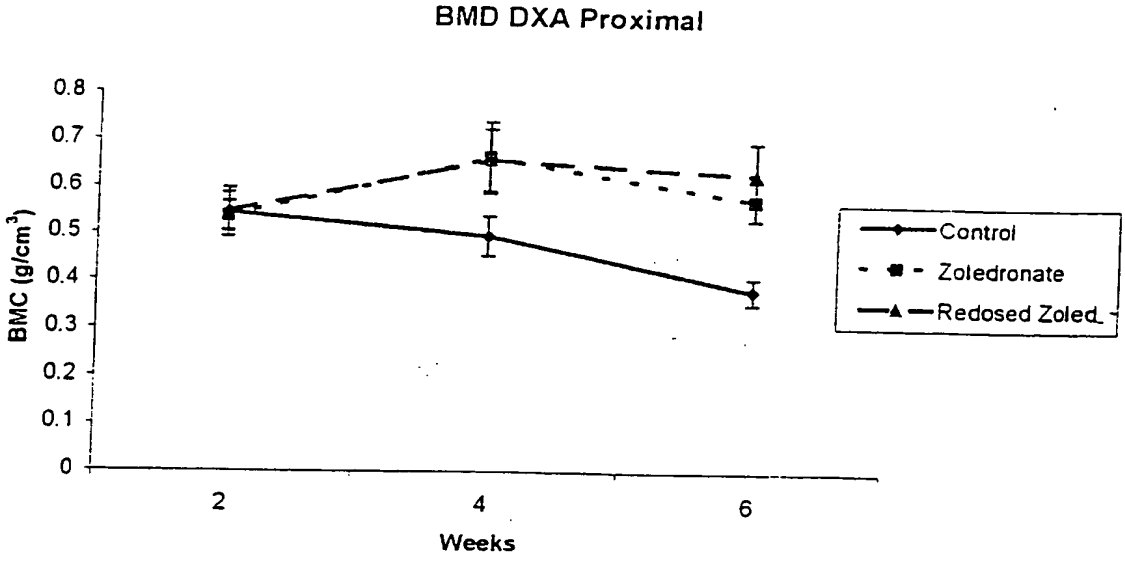

BMD DXA Proximal
BMC (g/cm3)
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
2
4
6
Weeks
Control
Zoledronate
Redosed Zoled

Figure 11B

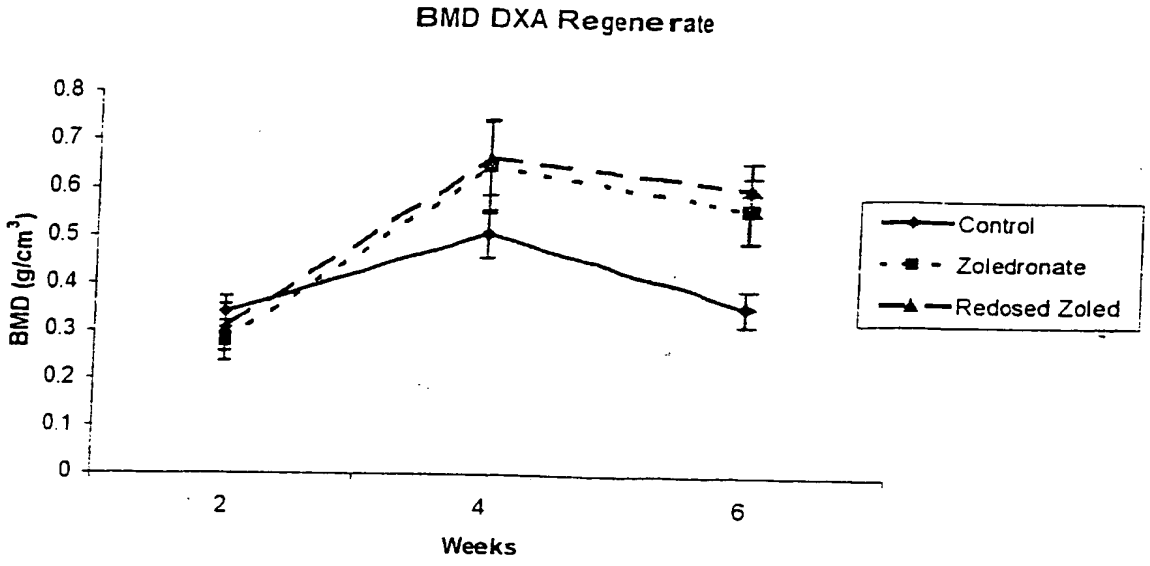

BMD DXA Regenerate
BMD (g/cm3)
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
2
4
6
Weeks
Control
Zoledronate
Redosed Zoled

Figure 11C

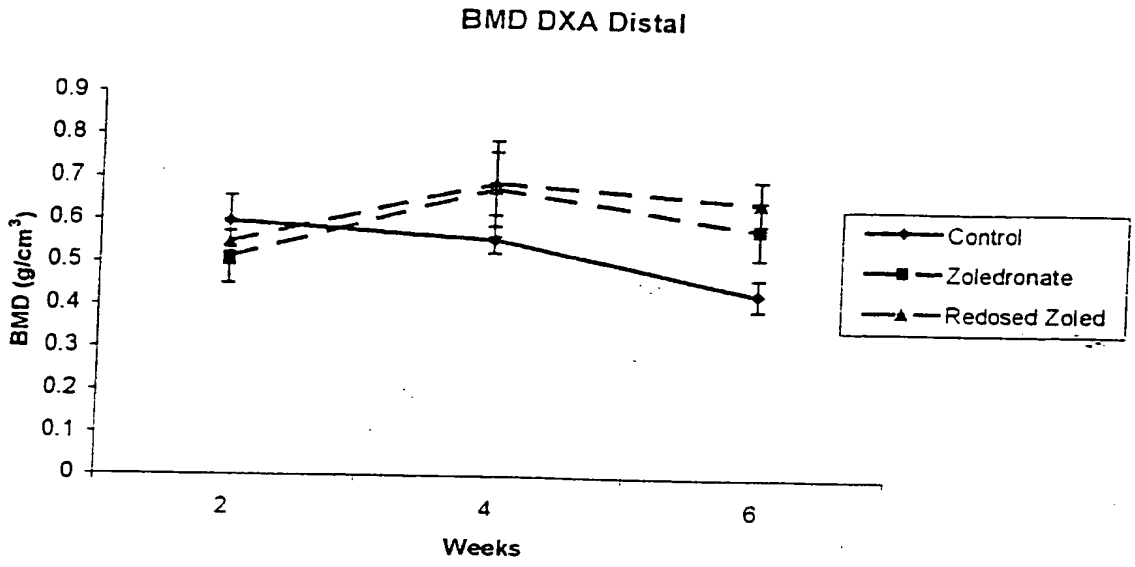

BMD DXA Distal
BMD (g/cm^3)
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
2
4
6
Weeks
Control
Zoledronate
Redosed Zoled

Figure 13

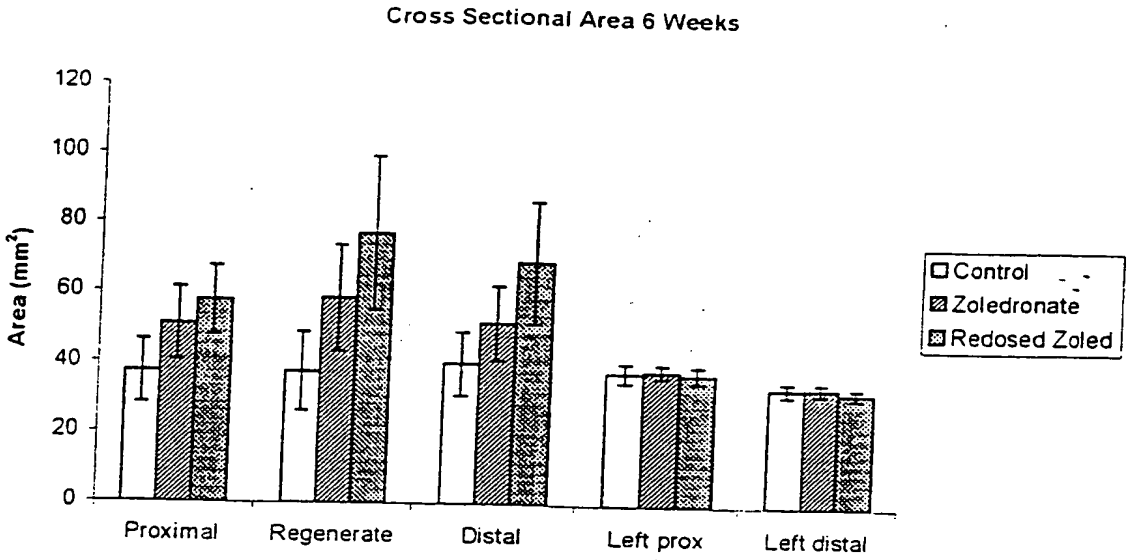

Cross Sectional Area 6 Weeks
Area (mm²)
120
100
80
60
40
20
0
Proximal
Regenerate
Distal
Left prox
Left distal
Control
Zoledronate
Redosed Zoled

Figure 14

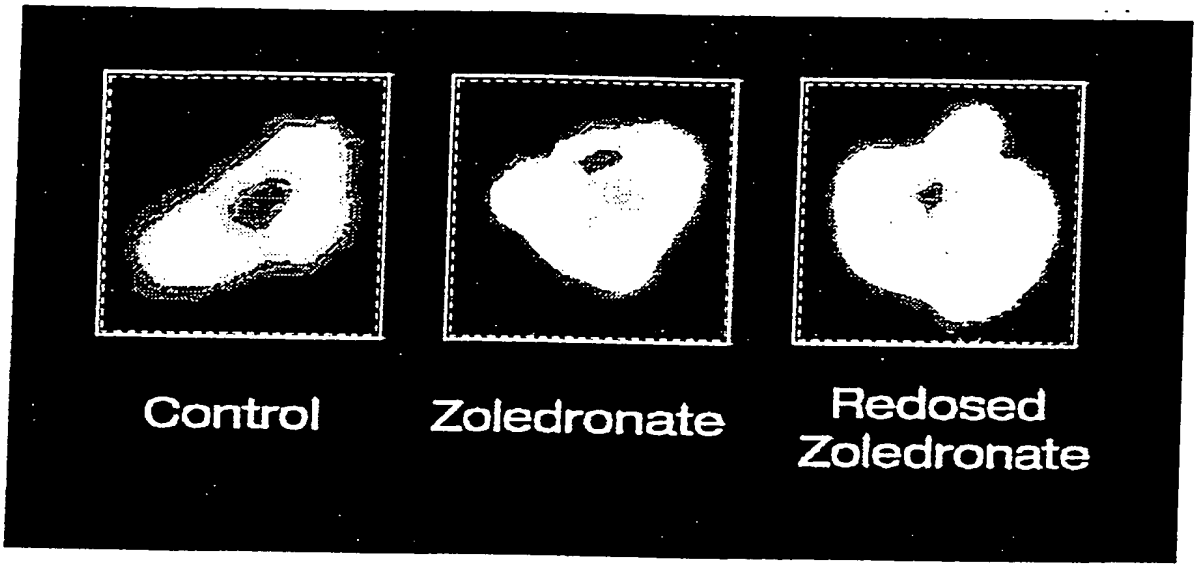
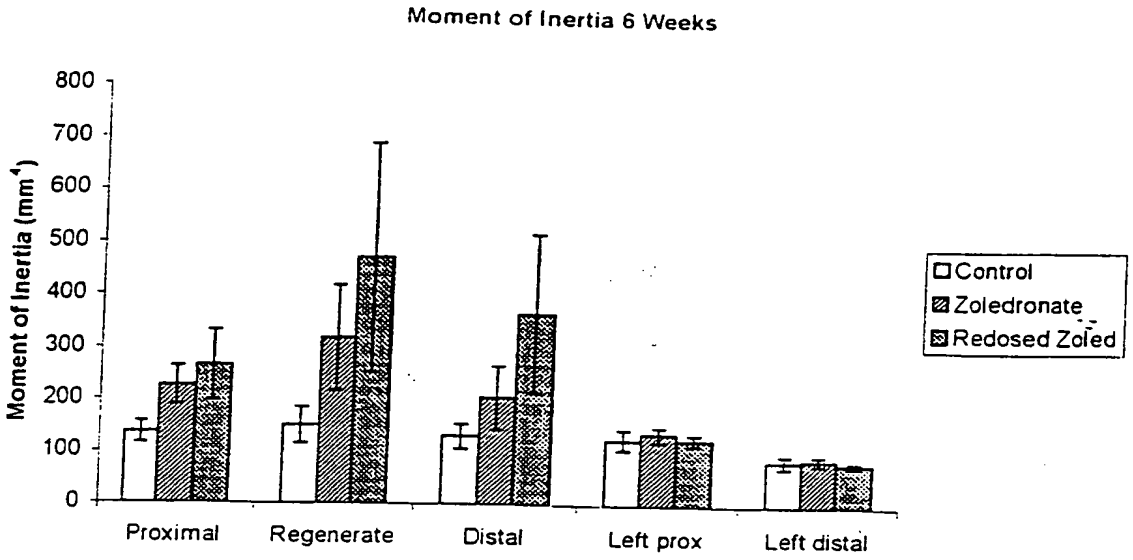


Figure 15


Moment of Inertia 6 Weeks
Moment of Inertia (mm⁴)
800
700
600
500
400
300
200
100
0
Proximal
Regenerate
Distal
Left prox
Left distal
Control
Zoledronate
Redosed Zoled

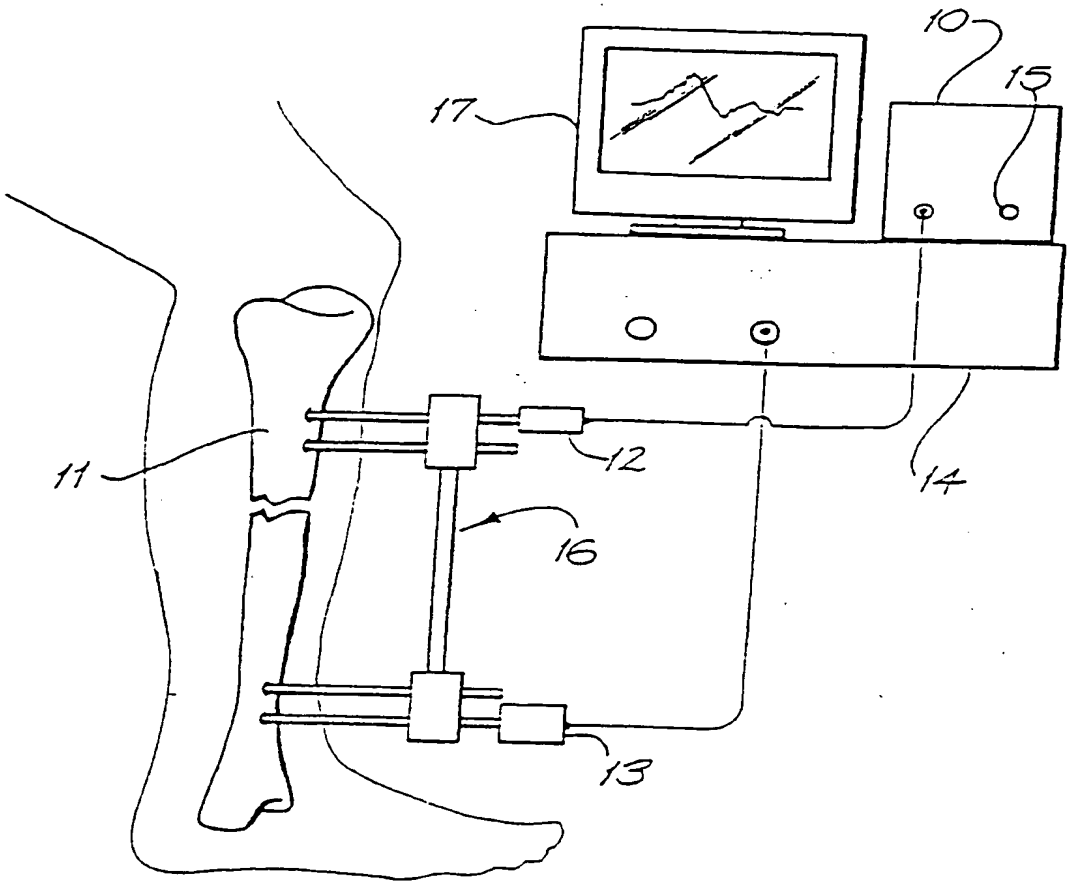


FIG. 16

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 101540	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416).
International Application No. PCT/AU00/00982	International Filing Date *(day/month/year)* 17 August 2000	Priority Date *(day/month/year)* 19 August 1999

International Patent Classification (IPC) or national classification and IPC

Int. Cl. [7] A61K 031/663, A61P 19/00

Applicant

THE ROYAL ALEXANDRA HOSPITAL FOR CHILDREN et al

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 3 sheets, including this cover sheet.

[X] This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

These annexes consist of a total of 5 sheet(s).

3. This report contains indications relating to the following items:

- I [X] Basis of the report
- II [ ] Priority
- III [ ] Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- IV [ ] Lack of unity of invention
- V [X] Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- VI [ ] Certain documents cited
- VII [ ] Certain defects in the international application
- VIII [ ] Certain observations on the international application

Date of submission of the demand	Date of completion of the report
23 November 2000	7 May 2001
Name and mailing address of the IPEA/AU AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA E-mail address: pct@ipaustralia.gov.au Facsimile No. (02) 6285 3929	Authorized Officer  **G.R.PETERS** Telephone No. (02) 6283 2184

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/AU00/00982

I. Basis of the report

1. With regard to the **elements** of the international application:*

☐ the international application as originally filed.

☒ the description, pages **1-4, 7-27**, as originally filed,
pages , filed with the demand,
pages **5, 6,** received on **30 April 2001** with the letter of **26 April 2001.**

☒ the claims, pages , as originally filed,
pages , as amended (together with any statement) under Article 19,
pages , filed with the demand,
pages **28-30,** received on **30 April 2001** with the letter of **26 April 2001.**

☒ the drawings, pages **1/20-20/20,** as originally filed,
pages , filed with the demand,
pages , received on with the letter of

☐ the sequence listing part of the description:
pages , as originally filed
pages , filed with the demand
pages , received on with the letter of

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.
These elements were available or furnished to this Authority in the following language which is:

☐ the language of a translation furnished for the purposes of international search (under Rule 23.1(b)).

☐ the language of publication of the international application (under Rule 48.3(b)).

☐ the language of the translation furnished for the purposes of international preliminary examination (under Rules 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, was on the basis of the sequence listing:

☐ contained in the international application in written form.

☐ filed together with the international application in computer readable form.

☐ furnished subsequently to this Authority in written form.

☐ furnished subsequently to this Authority in computer readable form.

☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

4. ☐ The amendments have resulted in the cancellation of:

☐ the description, pages

☐ the claims, Nos.

☐ the drawings, sheets/fig.

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).**

* *Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17).*

** *Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report*

V. **Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. Statement

Novelty (N)	Claims **1-36**	**YES**
	Claims	**NO**
Inventive step (IS)	Claims **1-36**	**YES**
	Claims	**NO**
Industrial applicability (IA)	Claims **1-36**	**YES**
	Claims	**NO**

2. Citations and explanations (Rule 70.7)

The new set of claims is directed to the use of bisphosphonates for the promotion of new bone formation at a fracture site and the use specifically of Zoledronate for promoting new bone formation (claim 5). The new claims are considered to be both novel and inventive.

The closest prior art is WO 94/21266, this document relates to the use of a certain group of bisphosphonates for the regeneration of bone tissues and/or elimination of late complications subsequent to bone surgery. Particularly, the invention concerns the use of clodronate to enhance bone tissue formation after surgical replacement of endo-osteal material such as implantations and transplantations. There is no disclosure in the citation of the use of bisphoshonates for treating bone fractures, there is also no disclosure in the citation of the use of the drug Zoledronate for promoting new bone formation.

(vi) Improving the ability of bone to support internal fixation devices in osteoporotic individuals or in locally osteoporotic bone segments

(vii) Treating fractures in which there are potential impediments to uncomplicated healing, for example:

(a) Fractures in the elderly including: neck of femur; supracondylar femur; tibia; ankle; humerus; and the distal radius (note that this list merely provides examples, and the use of bisphosphonates according to this invention is not by any means limited to treating these fractures only, or any other fractures, for that matter, in people of all ages)

(b) Pubic rami fatigue fracture

(c) Pathological fracture

(d) Scaphoid fracture

(e) Open fracture

(f) Fracture with periosteal disruption

(viii) Treating fractures that require prolonged immobilisation when treated non-operatively, for example: femoral fractures, tibial fractures; and fractures of the foot and ankle.

Further preferred embodiments also disclose a number of additional indications for using bisphosphonates in orthopaedic procedures. These include administering bisphosphonates to increase ingrowth of bone into joint replacement prostheses; and coating joint prosthesis with bisphosphonates to enhance the latter mentioned ingrowth at a more local level. Such therapy should also reduce the effects of periprosthetic stress shielding. Prosthetic implants may be so coated as an alternative, or in addition to coating with hydroxyapatite or some other osteoinductive coating.

One preferred embodiment discloses that the drug chosen from the class of bisphosphonates for carrying out this invention is Pamidronate. Another preferred embodiment discloses that the drug chosen from the group is Zoledronate. However, in further preferred embodiments, other bisphosphonates may be used in addition (where no adverse interaction results), or as an alternative, to Pamidronate or Zoledronate. Examples of further bisphosphonates include, but are not limited to, Alendronate, Tiludronate, Risedronate, Ibandronate and Incadronate.

In further preferred embodiments, the drug is administered to a

patient as a single dose. In this embodiment, it is preferred that the administration of the drug occurs early during the course of treating the fractured bone as administration of a bisphosphonate at such an early stage has a positive effect on the stimulation and proliferation of osteoblasts.

In a further embodiment, subsequent additional doses may be administered to the patient. In this embodiment, it is envisaged that a response to the first dose would be assessed before administering additional doses.

In still further preferred embodiments, the mode of administration may be as a perioperative intravenous infusion, orally, transdermally or by some other route. Alternatively a course of an oral bisphosphonate may be prescribed. All preferred and alternative embodiments of the invention envisage current and future available modes of administration for the drug. Such modes of administration must, of course be plausible, convenient and provide the patient with a therapeutically effective dose for treating and/or promoting healing of the fractured bone.

The present invention also discloses that in some embodiments, it is preferable to additionally apply a vibratory stimulus to the fractured bone. The vibratory stimulus may be provided by ultrasound stimulation and vibration stimulation, or any other mechanism and/or device capable of providing vibratory stimulation. In some embodiments, the vibratory stimulus may be applied at any frequency which is considered to be effective in the treatment of a fractured bone. In preferred embodiments, however, the step of providing a vibratory stimulus includes periodically providing a vibratory stimulus at the resonant frequency of the bone, said resonant frequency being calculated as a function of the bone's vibratory response to

CLAIMS:

1. A drug selected from the group consisting of at least one bisphosphonate when used for promoting new bone formation at a fracture site.

2. A drug selected from the group consisting of at least one bisphosphonate when used for treating a fractured bone.

3. The drug of claim 1 or claim 2 wherein the bisphosphonate is Zoledronate.

4. The drug of claim 1 or claim 2 wherein the drug is a combination of two or more bisphosphonates.

5. The drug Zoledronate when used for promoting new bone formation.

6. The drug of claim 5 when used for promoting new bone formation between a bone and a prosthesis, bone fixation device or any other bone or dental implant.

7. The drug of any one of the preceding claims when administered to an individual as a single dose.

8. The drug of any one of the preceding claims when administered to an individual perioperatively.

9. Use of a drug selected from the group consisting of at least one bisphosphonate for the manufacture of a medicament for promoting new bone formation at a fracture site.

10. Use of a drug selected from the group consisting of at least one bisphosphonate for the manufacture of a medicament for treating a fractured bone.

11. Use of the drug of claim 9 or claim 10 wherein the drug is Zoledronate.

12. Use of the drug of claim 9 or claim 10 wherein the drug is a combination of two or more bisphosphonates.

13. Use of the drug Zoledronate for the manufacture of a medicament for promoting new bone formation.

14. A method for treating a fractured bone, the method including administering to a subject with a fractured bone a therapeutically effective amount of a drug selected from the group consisting of at least one bisphosphonate.

15. The method of claim 14 wherein the drug is administered to the subject as a single dose.

16. The method of claim 15 wherein the single dose of drug is

administered at an early stage of treatment of the fractured bone.

17. The method of claim 14 wherein the mode of administration is as a perioperative intravenous infusion.

18. The method of claim 14 wherein the mode of administration is oral.

19. The method of claim 14 wherein the mode of administration is transdermal.

20. A method of treating a fractured bone, the method including the steps of:

(a) administering to a subject with a fractured bone a therapeutically effective amount of a drug selected from the group consisting of at least one bisphosphonate; and

(b) providing a vibratory stimulus to the fractured bone.

21. The method of claim 20 wherein the vibratory stimulus is provided by ultrasound stimulation or vibration stimulation.

22. The method of claim 20 or claim 21 wherein the vibratory stimulus includes periodically providing a vibratory stimulus at the resonant frequency of the bone.

23. The method of claim 22 wherein the resonant frequency is calculated as a function of the bone's vibratory response to the vibratory stimulus.

24. The method of any one of claims 20 to 23 wherein the vibratory stimulus is provided at a late stage in the treatment of the fractured bone.

25. The method of any one of claims 20 to 23 wherein the step of providing a vibratory stimulus is concurrent with the step of administering a therapeutically effective amount of the drug.

26. The method of claim 25 wherein the vibratory stimulus is provided and the therapeutically effective amount of the drug is administered at an early stage in the treatment of a fractured bone.

27. A drug selected from the group consisting of at least one bisphosphonate when used for promoting new bone formation at a fracture site in an individual suffering from delayed union of a fracture.

28. A method for promoting new bone formation at a fracture site in a subject suffering from delayed union of a fracture, the method including administering to the subject a therapeutically effective amount of a drug selected from the group consisting of at least one bisphosphonate.

29. The method of claim 28 wherein the at least one bisphosphonate is administered parenterally as a single dose at or near the time of surgery.

30. The method of claim 29 wherein a further parenteral dose of the at least one bisphosphonate is administered about four to six weeks after the initial dose.

31. The method of claim 29 wherein further oral doses of the at least one bisphosphonate are administered in a daily or second daily regimen commencing about four to six weeks after the initial dose for a period of about two months or until sufficient new bone has been formed.

32. A method of promoting new bone formation in a subject, the method including the steps of surgically performing the procedure of distraction osteogenesis and administering to the subject a drug selected from the group consisting of at least one bisphosphonate.

33. The method of claim 32 wherein the at least one bisphosphonate is administered parenterally as a single dose at or near the time of surgery.

34. The method of claim 33 wherein a further parenteral dose of the at least one bisphosphonate is administered either at the end of the distraction period or up to three months after the initial dose.

35. The method of claim 33 wherein further oral doses of the at least one bisphosphonate are administered in a daily, second daily or weekly regimen.

36. The method of claim 35 wherein the regimen commences about one to three months after the initial parenteral dose for a period of about two months.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU00/00982

A. CLASSIFICATION OF SUBJECT MATTER

Int. Cl. [7] A61K 031/663, A61P 19/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K 031/66

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
AU:IPC AS ABOVE

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
WPAT, MEDLINE; keywords: bisphosphonate, Zoledronate, Pamidronate, bone, fracture, skelet:.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 98/00438A (THE UNIVERSITY OF LIVERPOOL) 8 January 1998. See whole document.	1-20
X	WO 96/39150A (MERCK & CO., INC) 12 December 1996. See whole document.	1-20
X	WO 96/39151A (MERCK & CO., INC) 12 December 1996. See whole document.	1-20.
X	WO 95/28936A (MERCK & CO., INC) 2 November 1995 . See whole document.	1-20

[X] Further documents are listed in the continuation of Box C [X] See patent family annex

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search
21 September 2000

Date of mailing of the international search report
26 SEP 2000

Name and mailing address of the ISA/AU

AUSTRALIAN PATENT OFFICE
PO BOX 200, WODEN ACT 2606, AUSTRALIA
E-mail address: pct@ipaustralia.gov.au
Facsimile No. (02) 6285 3929

Authorized officer

G.R.PETERS

Telephone No : (02) 6283 2184

Form PCT/ISA/210 (second sheet) (July 1998)

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/AU00/00982

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report	Patent Family Member				
WO 98/00438	AU 33506/97	BG 103123	BR 9710074	CN 122625	CZ 9804323
	EP 912598	GB 9613722	HU 9902880	ZA 9705744	
WO 96/391450	AU 59679/96	CA 2221416	EP 831843		
WO 96/39151	AU 60309/96	CA 2223400	EP 831844	IL 122149	US 5616571
WO 95/28936	AU 234748/95	BG 100910	CA 2188030	CN 1146152	EP 756483
	HU 9602888	NO 964441	US 5646134		
WO 94/21266	AU 62093/94	EP 689443	US 5403829		
WO 93/11786	AU 30226/92	BR 9206941	EP 618805	RU 2104699	US 5616560
	ZA 9209758				

END OF ANNEX

fusing of a joint to increase the fusion rate.

One preferred embodiment discloses that the drug chosen from the class of bisphosphonates for carrying out this invention is Pamidronate. Another preferred embodiment discloses that the drug chosen from the group is Zoledronate. However, in further preferred embodiments, other bisphosphonates may be used in addition (where no adverse interaction results), or as an alternative, to Pamidronate or Zoledronate. Examples of further bisphosphonates include, but are not limited to, Alendronate, Tiludronate, Risedronate, Ibandronate and Incadronate.

In further preferred embodiments, the drug is administered to a patient as a single dose. In this embodiment, it is preferred that the administration of the drug occurs early during the course of treating the fractured bone as administration of a bisphosphonate at such an early stage has a positive effect on the stimulation and proliferation of osteoblasts.

In a further embodiment, subsequent additional doses may be administered to the patient. In this embodiment, it is envisaged that a response to the first dose would be assessed before administering additional doses.

In still further preferred embodiments, the mode of administration may be as a perioperative intravenous infusion, orally, transdermally or by some other route. Alternatively a course of an oral bisphosphonate may be prescribed. All preferred and alternative embodiments of the invention envisage current and future available modes of administration for the drug. Such modes of administration must, of course be plausible, convenient and provide the patient with a therapeutically effective dose for treating and/or promoting healing of the fractured bone.

The present invention also discloses that in some embodiments, it is preferable to additionally apply a vibratory stimulus to the fractured bone. The vibratory stimulus may be provided by ultrasound stimulation and vibration stimulation, or any other mechanism and/or device capable of providing vibratory stimulation. In some embodiments, the vibratory stimulus may be applied at any frequency which is considered to be effective in the treatment of a fractured bone. In preferred embodiments, however, the step of providing a vibratory stimulus includes periodically providing a vibratory stimulus at the resonant frequency of the bone, said resonant frequency being calculated as a function of the bone's vibratory response to

(vi) Improving the osteogenic potential of bone graft – autologous graft, allograft or synthetic bone graft substitute

(vii) Improving the ability of bone to support internal fixation devices in osteoporotic individuals or in locally osteoporotic bone segments

(viii) Treating fractures in which there are potential impediments to uncomplicated healing, for example:

(a) Fractures in the elderly including: neck of femur; supracondylar femur; tibia; ankle; humerus; and the distal radius (note that this list merely provides examples, and the use of bisphosphonates according to this invention is not by any means limited to treating these fractures only, or any other fractures, for that matter, in people of all ages)

(b) Pubic rami fatigue fracture

(c) Pathological fracture

(d) Scaphoid fracture

(e) Open fracture

(f) Fracture with periosteal disruption

(ix) Treating fractures that require prolonged immobilisation when treated non-operatively, for example: femoral fractures, tibial fractures; and fractures of the foot and ankle.

(x) Treatment of patients with avascular necrosis to enhance new bone formation and prevent collapse

(xi) Treatment of congenital pseudarthrosis of the tibia and related conditions.

Further preferred embodiments also disclose a number of additional indications for using bisphosphonates in orthopaedic procedures. These include administering bisphosphonates to increase ingrowth of bone into joint replacement prostheses; and coating joint prosthesis with bisphosphonates to enhance the latter mentioned ingrowth at a more local level. Such therapy should also reduce the effects of periprosthetic stress shielding. Prosthetic implants may be so coated as an alternative, or in addition to coating with hydroxyapatite or some other osteoinductive coating.

Furthermore, bisphosphonates may be used in arthrodesis, that is,

CLAIMS:

1. A drug selected from a group consisting of at least one bisphosphonate when used for promoting bone growth.

2. The drug of claim 1 when used for promoting bone growth at a fracture site.

3. The drug of claim 1 or claim 2 when used for promoting bone growth between a bone and a prosthesis, bone fixation device or any other bone or dental implant.

4. A drug selected from a group consisting of at least one bisphosphonate when used for treating a fracture.

5. The drug of any one of the preceding claims wherein the bisphosphonate is Zoledronate.

6 The drug of any one of claims 1 to 4 wherein the bisphosphonate is Pamidronate.

7. The drug of any one of claims 1 to 4 wherein the drug is a combination of two or more bisphosphonates.

8. Use of a drug selected from the group consisting of at least one bisphosphonate for the manufacture of a medicament for promoting bone growth.

9. Use of the drug of claim 8 for the promotion of bone growth at a fracture site.

10. Use of the drug of claim 8 for the promotion of bone growth between a bone and a prosthesis.

11. Use of a drug selected from a group consisting of at least one bisphosphonate for the manufacture of a medicament for treating a fractured bone.

12. Use of the drug of any one of claims 8 to 11 wherein the drug is Zoledronate.

13. Use of the drug of any one of claims 8 to 11 wherein the drug is Pamidronate.

14. Use of the drug of any one of claims 8 to 11 wherein the drug is a combination of two or more bisphosphonates.

15. A method for treating a fractured bone, the method including administering to a subject with a fractured bone a therapeutically effective amount of a drug selected from a group consisting of at least one bisphosphonate.

16. The method of claim 15 wherein the drug is administered to the subject as a single dose.

17. The method of claim 16 wherein the single dose of drug is administered at an early stage of treatment of the fractured bone.

18. The method of claim 15 wherein the mode of administration is as a perioperative intravenous infusion.

19. The method of claim 15 wherein the mode of administration is oral.

20. The method of claim 15 wherein the mode of administration is transdermal.

21. A method for treating a fractured bone, the method including the steps of:

(a) administering to a subject with a fractured bone a therapeutically effective amount of a drug selected from a group consisting of at least one bisphosphonate; and

(b) providing a vibratory stimulus to the fractured bone.

22. The method of claim 21 wherein the vibratory stimulus is provided by ultrasound stimulation or vibration stimulation.

23. The method of claim 21 or claim 22 wherein the vibratory stimulus includes periodically providing a vibratory stimulus at the resonant frequency of the bone.

24. The method of claim 23 wherein the resonant frequency is calculated as a function of the bone's vibratory response to the vibratory stimulus.

25. The method of any one of claims 21 to 24 wherein the vibratory stimulus is provided at a late stage in the treatment of the fractured bone.

26. The method of any one of claims 21 to 24 wherein the step of providing a vibratory stimulus is concurrent with the step of administering a therapeutically effective amount of the drug.

27. The method of claim 26 wherein the vibratory stimulus is provided and the therapeutically effective amount of the drug is administered at an early stage in the treatment of a fractured bone.

28. The method of claim 24 wherein the vibratory stimulus is provided and the therapeutically effective amount of at least one drug from the class of bisphosphonates is administered at a late stage in the treatment of a fractured bone.